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Resumen por el autor, Ross G. Harrison,
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Sohre las relaciones de simetría en los miembros transplantados.

El autor ha llevado a cabo los siguientes experimentos con los esbozos de los miembros anteriores de Amblystoma: Transplante a la superficie lateral del cuerpo de otro embrión; transplante al sitio normal después de extirpar el esbozo del miembro que le ocupaba; superposición de un esbozo sobre otro después de separar su ectodermo; y transplante de medio esbozo en el sitio en que existia otro medio esbozo, extirpado previamente. Los injertos fueron obtenidos en el mismo lado del cuerpo o en el lado opuesto e implantados con el eje dorso-ventral situado normalmente o invertido. Los dos primeros grupos de experimentos indican que cuando el eje dorso-ventral del miembro no está invertido persiste la simetría prospectiva originaria, y que cuando se invierte, la simetría se invierte también (simetria enantiomórfica). La asimetría está determinada: 1) Por la polarización del eje antero-posterior del esbozo y 2) Por la orientación del esbozo respecto a la polarización dorso-ventral del ambiente orgánico. Dos combinaciones (armónicas) producen miembros con la asimetría correspondiente al lado en que se colocaron; otras dos (desarmónicas) producen miembros con asimetría opuesta. Se encuentran con frecuencia reduplicaciones el miembro primario sigue en este caso las reglas mencionadas, mientras que los miembros secundarios simulan sus imágenes producidas por un espejo, y a veces duplicados a su vez. Existe conformidad con las reglas de Bateson, las cuales sin embargo pueden enunciarse mas simplemente para incluir los supernumerarios sencillos y dobles. En los experimentos con mitades de esbozos superpuestas, salvo ciertas excepciones, las combinaciones armónicas producen miembros sencillos y las desarmónicas reduplicaciones de acuerdo con las reglas. El mesodermo del esbozo del miembro es un "sistema armónico equipotencial" y excepto para la determinación de ciertas relaciones axiales, es autodiferenciable. Su forma, incluso sus relaciones simétricas, debe estar representada en su estructura íntima.

ON RELATIONS OF SYMMETRY IN TRANSPLANTED LIMBS

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ONE HUNDRED AND THIRTY-SIX FIGURES

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1

INTRODUCTION

The circumstance that originally suggested the present study was the apparent difference in the results obtained by Streeter ('07) and by Spemann ('10) in their respective experiments with the amphibian ear vesicle. According to the original account of Streeter, the otocyst, when taken out of an embryo just after closure and replaced after having been rotated 180° on any of its axes, develops in normal posture, though a right vesicle placed on the left side remains true to its side of origin. According to Spemann, the inverted vesicle develops in inverted position, the rudiments of the constituent parts being localized, at the time of operation. Although subsequent work by Streeter ('14) seems to have shown that the normal development of the inverted vesicles, found in his cases, was due to their regaining normal posture by rotation as a whole, the original divergence of results nevertheless had raised theoretical questions of great interest, which Spemann has ably discussed. The main question was whether we might have in the otic vesicle an 'harmonic equipotential system' with its future asymmetry in some way stamped upon its intimate structure. Though Spemann's analysis answers the question in the negative, as far as the closed ear vesicle is concerned, it is nevertheless important to determine how far, if at all, systems of this kind are present in the embryo, for their study would throw light upon the question of the mode of representation of adult form characters in the germ, giving evidence from a new quarter with regard to the great problems of development which have usually been approached by way of experiments upon the unsegmented egg and the early stages of cleavage. The method of embryonic transplantation obviously

affords a means of studying this question in any organ or part that in the adult lacks a plane of symmetry. It was with this purpose in view that the present experiments with the limbs of Amblystoma were begun. Limb buds were implanted in both normal and abnormal location, oriented in various ways with respect to the main axes of the embryo-host, and the form and posture of the resulting limbs were studied.

It became evident, after the first experiments were made, that the rudiment of the fore limb behaved differently from the auditory vesicle, no matter whether Streeter's original interpretation or Spemann's was accepted as correct. While it was found that a certain tendency did exist for inverted limb buds to rotate back to normal posture during development, this was not the usual result, nor did the rotation take place in the sense meant by Streeter in his later publication ('14). Furthermore, many irregularities of development were produced by the operations, due largely to the power of the limb rudiment to duplicate itself by budding.[1] On the other hand, it often occurred that buds transplanted from one side of the body to the other developed in harmony with their new surroundings, a right limb bud, for example, placed on the left side, giving rise to a normal left limb.

The earlier experiments which were made in 1911 and 1912 led to no satisfactory general conclusion, so that publication of the work was deferred pending further investigation. Subsequently numerous additional experiments were made, in which more effective precautions against regeneration of the limbs from the host were taken.[2] The situation began to clear when in some of the cases in which the asymmetry of the limb was reversed by

[1] Cf. Barfurth ('94), who showed that supernumerary limbs could be produced in amphibians by regeneration after irregular amputation; Tornier ('05), who obtained multiple appendages by cutting into the limb bud of tadpoles; Braus ('04, '05, and '09) and Harrison ('07), who found that transplanted limb buds frequently give rise to double limbs.

[2] The first experiments were reported to the National Academy of Sciences in November, 1912, at the New Haven meeting. Later reports were made before the American Association of Anatomists in December, 1915 ('16), and before the American Society of Zoologists in the following year ('17 a). The main results have been stated somewhat more fully in the Proceedings of the National Academy ('15 and '17 b).

transplantation it was observed that the reversal came about
by a process of reduplication or twinning. By following closely
the history of individual cases, it became evident that the double
formation was not infrequently obscured by the preponderance
of the reduplicating limb bud over the original, so that the former
grew into a member of opposite asymmetry, while the original
bud was reduced to a mere spur or nodule, which might readily
be overlooked. The tendency to produce duplicities thus proved
to be even greater than the actual number of fully developed
cases indicated. In other cases the reversal appeared to be more
direct; at least, a limb of the side of origin often failed to appear
as such on the surface, though slight irregularities in the early
stages of development, coupled with an appreciable delay in the
process, showed that some internal readjustment of the grafted
tissue was taking place.

It seemed that the functional activity of the limb, when trans-
planted to its normal environment, might accentuate the appar-
ently anomalous results just described. In order to eliminate
this factor, a series of experiments in which the limb bud was
grafted on some other part of the body was undertaken. Here
the proper nervous connections did not become established, func-
tional activity was usually lacking or was at best but slightly
developed, and the undisturbed effect, upon development, of the
relative orientation of the tissues of graft and host could be ob-
served. The latter experiments led to the formulation of the
three following simple rules,[3] which hold for implantations either
in normal or in abnormal location:

Rule 1. A bud that is not inverted (dorsodorsal) gives rise to
a limb of the side of origin of the bud, whether implanted on the
same or on the opposite side of the body.

Rule 2. An inverted bud (dorsoventral) gives rise to a limb
of reversed asymmetry, whether implanted on the same or on
the opposite side of the body.

Rule 3. When double limbs arise, the original one (the one
first to begin its development) has its asymmetry fixed in accord-

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[3] These rules were phrased somewhat differently in two preliminary communi-
cations ('17 a and '17 b).

ance with rule 1 or 2, while the other is the mirror image of the first.

Experiments previously reported[4] have shown that the limb bud is an 'harmonic equipotential system,'[5] and additional experiments with inverted buds (p. 87) and with half buds (p. 83) confirm this result. We must assume, then, that the potencies of the cells of the limb bud to form the fore limb are in the last instance represented in their intimate structure and not merely in their arrangement. The above rules show, however, that not all essential features are stamped upon the constituent elements of the rudiment at the time of transplantation. For example, the difference between the right bud and the left is not an absolute one, since a right limb bud upside down behaves like a left one right side up and vice versa. From this the conclusion has been drawn that the elements making up the limb bud are differentiated in an anteroposterior direction, i.e., along the anteroposterior axis, but are not yet differentiated, at least not irreversibly, along the dorsoventral axis at the period of development at which the transplantations are made. In this one respect the differentiation of the limb is dependent upon its orientation with reference to the dorsoventral axis of the embryo; otherwise as regards its specific form, the limb bud constitutes a self-differentiating system.

These questions will be considered more fully in the concluding section (p. 85).

METHODS AND TERMINOLOGY

All experiments were made upon embryos of Amblystoma punctatum in stages that have been previously defined.[6]

In performing the operations the embryo which is to receive the implanted limb bud is first made ready. If the bud is to be placed in normal location, the wound is prepared as in the extirpation experiments referred to above. A circular incision, hav-

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[4] Harrison, '18.

[5] "Jedes kann Jedes und alles Einzelne steht in Harmonie zu einander." (Driesch, '02, p. 229.) See also: '99, p. 72; '05, p. 679, and '08 b, p. 120.

[6] Harrison, '15 and '18.

ing the diameter of three and a half somites (ca. 0.9 mm.) and ventral to the third, fourth, fifth, and half of the sixth myotome, is made, and the disc of tissue, including both mesoderm and ectoderm, is lifted, after which the remaining mesoderm cells are carefully cleaned off. This may all be done without injury to the pronephros, which lies immediately dorsal to the limb rudiment, though if this organ is injured or even extirpated, there is no noticeable effect on the subsequent development of the limb. The embryo thus prepared is held in readiness for the grafting, being secured in position by pieces of silver wire or glass rod bent into proper shape. The limb bud which is to be transplanted is removed from another embryo, as described above, care being taken in lifting it from its bed to take all of the mesoderm possible. It is then transferred on the point of the scissors to the first embryo and fitted into the wound. The orientation of the bud is important and may be carried out as desired by noting the position of pigment markings in the ectoderm of the graft. After it has been properly placed, it is held in position for an hour or more by a single piece of glass rod, bent into such shape that it straddles the embryo, exerting a light pressure upon the grafted tissue. The healing of the wound takes place readily, though frequently a small area of underlying yolk may be left exposed on the border of the wound. This usually heals in a day or two and does not seem to influence the result of the experiment, unless the yolk begins to disintegrate, in which case death of the embryo usually follows.

At the time when the first experiments were made, the conditions necessary to prevent regeneration had not been determined, so that in a number of cases the extirpated area was too small or the wound bed was insufficiently cleaned of scattered mesoderm cells for the result to be conclusive. These experiments have not been included in the tabulations, but will be referred to separately in so far as they are of special interest. In all of the later experiments the size and character of the wound in the host was such as to preclude regeneration of the limb from that source; the resulting limbs must, therefore, have arisen from the engrafted tissue. Even in the cases where the wound was

not especially cleaned, there is no evidence, aside from certain exceptional cases, that the tissue of the wound bed displaces the transplanted bud, though the possibility of its participation in the make-up of the limb cannot be excluded, and it probably actually does take place to some extent.

In transplanting the limb bud to a location other than the normal, the recipient embryo is first prepared as in the other experiments. A wound of proper size is made, usually in the flank just below the ventral border of the myotomes, and the bud grafted in the same manner as described above. In doing this it is well to avoid injury to the pronephric duct (p. 15).

Three different factors regarding the placement of the limbs have been considered in these experiments, viz.: 1) location of the graft in the embryo; 2) the side of the body on which it is placed (whether the same from which it was taken or the opposite); and 3) orientation with respect to the cardinal points of the embryo. The experiments thus fall into eight categories, as follows (fig. 1):

A. Limb buds placed in abnormal location—heterotopic transplantation.

1. On the same side of body as origin—homopleural (*hom.*).

a. Dorsal border of limb bud dorsal with respect to embryo— dorsodorsal (*dd.*).

b. Dorsal border of limb bud ventral with respect to embryo— dorsoventral (*dv.*).

2. On side of body opposite to origin—heteropleural (*het.*).

a. Dorsal border of limb bud dorsal with respect to embryo— dorsodorsal (*dd.*), in which case the anteroposterior axis is reversed.

b. Dorsal border of limb bud ventral with respect to embryo— dorsoventral (*dv.*), in which case the anterior and posterior points of the graft correspond, respectively, to those of the embryo.

B. Limb bud placed in natural location—orthotopic transplantation.

The several categories under this head as under A.

According to the rules stated on page 4, two of the combinations (homopleural dorsodorsal and heteropeural dorsoventral) yield limbs which are of the same side of the body as that on

which they are placed (fig. 2), for the non-inverted limb bud from the same side (*hom.dd.*) does not have its prospective asymmetry changed while the inverted limb bud from the opposite side (*het.dv.*) does. The limbs which develop in these combina-
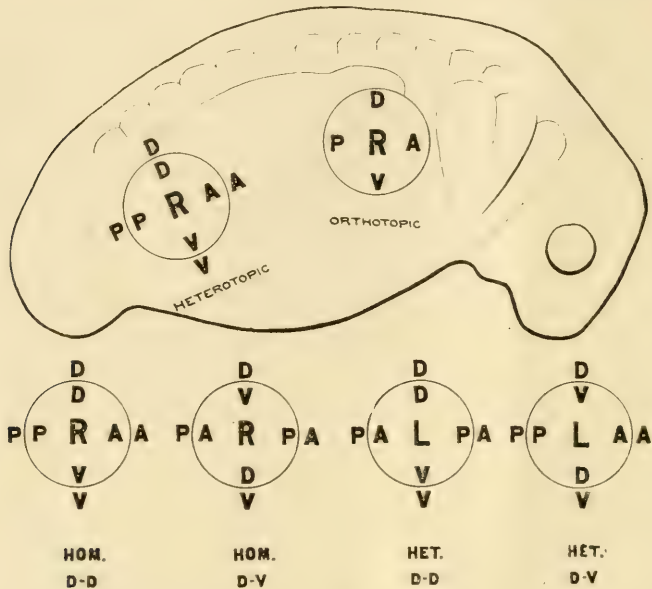
Fig. 1 Diagram showing the eight different operations. The outline of an Amblystoma embryo in the operating stage is shown above. The circles within it represent the limb bud, in the normal (orthotopic) and the abnormal (heterotopic) location. The four circles below represent the four different ways in which limb buds may be oriented with reference to the cardinal points of the embryo; the letters (*D*, dorsal; *V*, ventral; *A*, anterior, and *P*, posterior) within the circles designate the original cardinal points of the transplanted limb, those outside the corresponding points of the embryo. The operations are represented to be on the right side. *R*, right limb bud; *L*, left limb bud; *hom.*, homopleural; *het.*, heteropleural.

tions thus fit in with their surroundings; they have therefore been called harmonic. The other two combinations (homopleural dorsoventral and heteropleural dorsodorsal) give rise to limbs of the side opposite to that of their seat of implantation, for the inverted bud from the same side (*hom.dv.*) has its asymmetry reversed, while the non-inverted bud from the opposite side

(*het.dd.*) remains as it was. The limbs which develop here are not primarily in harmony with their surroundings, so that these combinations have been termed disharmonic.
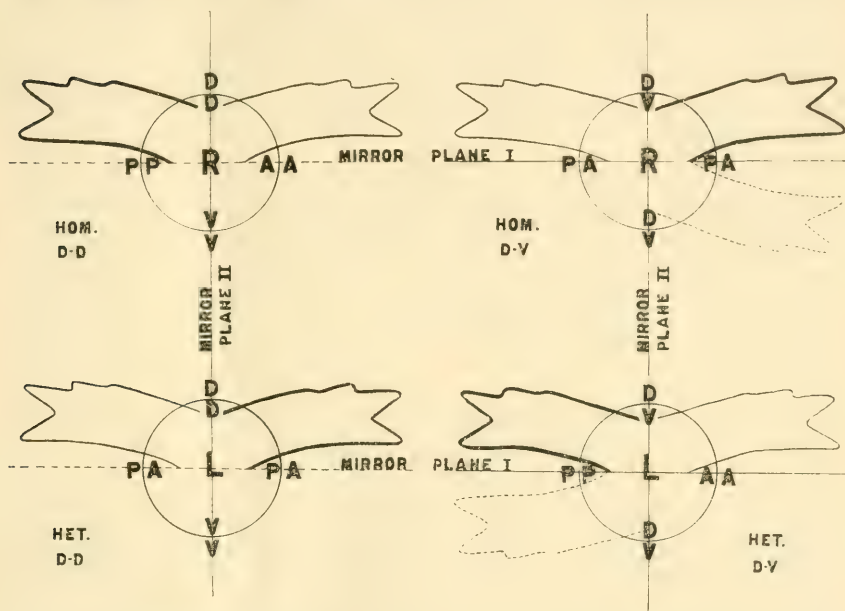
Fig. 2 Diagram showing the results of the four operations, heterotopic or orthotopic, represented as on the right side of the embryo. The circles indicate the transplanted limb buds, the letters having the same significance as in figure 1. Thus the two upper figures in the diagram represent homopleural, and the two lower ones heteropleural transplantations. The two on the left show the transplanted bud in upright (dorsodorsal) orientation while the two on the left are inverted (dorsoventral). The limbs which develop are shown in profile, the ulnar border being uppermost (dorsal) in all which actually develop. A heavy outline indicates the primary member, a light outline the reduplicating one. It is to be noted, however, that the latter develop in by no means all cases, while the former may be resorbed in the heteropleural dorsoventral combination, leaving only the reduplicating member present. The broken outlines show the posture that the limb would have assumed, had it developed as a self-differentiating member totally independent of the influence of its surroundings.

The transplanted limb bud is a flattened disc of tissue, and it is theoretically possible to make eight further combinations by placing the medial surface of the graft corresponding to the lateral surface of the embryo. This is impracticable, however, because

the mesoderm would thus be brought to the surface and the ecto-
derm buried beneath it. The same effect might be obtained,
however, by transplanting the mesoderm alone. While the dif-
ficulties in this procedure are great, they have now been in a large
measure overcome. The positive experiments are too few in
number to warrant any very definite statement, but they do indi-
cate that it is immaterial which surface of the mesodermal disc
faces laterally.

A much greater variety of experiments could be had by experi-
menting with positions intermediate between the upright and
inverted positions, i.e., with limb buds turned, say, 90° instead
of 180°. Such experiments may yield very interesting results,
but as yet there has not been sufficient time to carry them out,
nor has the effect of implanting the limb exactly in the midline
been studied.

The experiments with superposed buds were made in the same
way as the above, except that the mesoderm of the host was not
excised. In the case of half buds, more combinations are pos-
sible, as described in the section dealing with this group. Both
here and in the superposition experiments all possible positions
with regard to the placement of the graft within the limitations
stated above were experimented with. Relations of harmony and
disharmony proved to be the same here as in the case of whole
buds.

The total number of cases of which records have been kept is
462. The analysis is based, however, upon the 271 individuals
which yielded positive results. The identity of the individual
cases has been maintained by rearing each in a container by
itself and keeping a separate history of each. These histories
consist in notes and in sketches made from time to time directly
from the living specimens, mostly with the aid of the camera
lucida.

In dealing with so large a mass of material it has of course been
necessary to select typical cases for presentation, and in order
not to interrupt the continuity of the general account, the indi-
vidual histories, as far as given, have been gathered together in
an appendix. The main body of the paper has been divided in

accordance with the outline presented above. The larger groups of experiments have been considered apart from each other, and each subgroup is treated in a special section. The peculiar features of each of the larger groups have been considered at the beginning, and the results of the experiments summarized separately at the end of each main section. The more general questions are treated in the final chapter.

It has been thought best to provide numerous illustrations in order to avoid lengthy descriptions. Since it was not possible to keep a complete pictorial history of each case, those were selected for drawing that promised typical or otherwise interesting results. Unfortunately, however, it was not always possible to predict what the outcome of an experiment would be, so that some important cases were not drawn in early stages, while others of less interest were.[7]

GENERAL FEATURES OF THE DEVELOPMENT OF THE TRANSPLANTED BUDS

The development of the transplanted limb buds must now be considered in comparison with normal development. When the normal limb bud appears it is a round prominence just below the pronephros. It soon becomes more sharply marked off from the background and begins to 'point' dorsoposteriorly.[8] The radial border of the fore arm and hand is at first ventrolateral, then ventral, and the first digits to arise are the first and second. The third and fourth digits appear later on the dorsal border of the hand, so that there is never any difficulty in distinguishing the ulnar from the radial border unless the third and fourth digits are entirely suppressed. The palmar surface of the hand faces at first ventromedially and later medially.

The transplanted limbs, both heterotopic and orthotopic, give evidence of their orientation early in development, inasmuch

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[7] Almost all of the preliminary sketches and many of the finished drawings were made by Miss Lisbeth Krause. The former, which were pencil sketches, had to be redrawn for reproduction. For this part of the work and also for a number of the original drawings I am indebted to Mr. A. Hemberger and Mr. H. D. Rhynedance.

[8] Harrison, '18, p. 419.

as their direction of 'pointing' is determined principally by the
bud itself.   In two of the combinations (homopleural dorsoven-
tral and heteropleural dorsodorsal), they point anteriorly or
dorsoanteriorly; in the other two (homopleural dorsodorsal and
heteropleural dorsoventral) posteriorly or dorsoposteriorly like
the normal.   The subsequent development in the latter case is
normal, but in the former there is a tendency for the limb to stick
out more sharply to the side or to rotate more or less from the
position in which it would be found were the position determined
entirely by the orientation of the bud itself.   Nevertheless, the
palm tends to face ventromedially, or else the limb is so rotated
that it faces more ventrally or anteriorly.   In order to determine
whether the limb is right or left, it is necessary to be able to dis-
tinguish between the palm and the back of the hand, which is
not always so simple as it might seem.   It can usually be done,
however, by noting the digits, which are frequently slightly
flexed.   When there is uncertainty, it is necessary to resort to
sections, in which case there is no difficulty in distinguishing be-
tween the two faces, because of the much greater thickness of
the soft parts on the flexor surface of the skeleton.

The duplicities that arise are of all grades and kinds, and occur
in very different proportions in the several experiments.   Some-
times they make their appearance very early, sometimes late in
development.   In the orthotopic grafts reduplication is far more
common when the developing limb and the substratum are of
opposite sides.   In such cases the doubling member nearly always
appears as a bud posterior to the main limb, growing there into
a limb of proper asymmetry.   The extent of reduplication may
include the whole limb from the shoulder down, or only certain
of the digits.   The duplicate limb is as if it were mirrored from
the original in a plane which is perpendicular to the plane of the
proximodistal axes of the two limbs[9] and which cuts the axes
of the two limbs at their junction, at an angle which varies from
almost 0° to 90°.   In the former case the two members are
almost parallel, in the latter they diverge in the opposite direction
at almost 180°, the mirror plane bisecting the angle between them

[9] Bateson, Materials for the Study of Variation, p. 479.

(fig. 3).   In the present paper the relation of the mirror plane
to the long axis of the limb has not been taken into account for
purposes of description, the relation only to the dorsopalmar and
the radioulnar axes being stated; i.e., the degree of divergence
of the two members is not taken into account.   Thus, when the
mirror plane is parallel to the radioulnar axis, the limb is said to
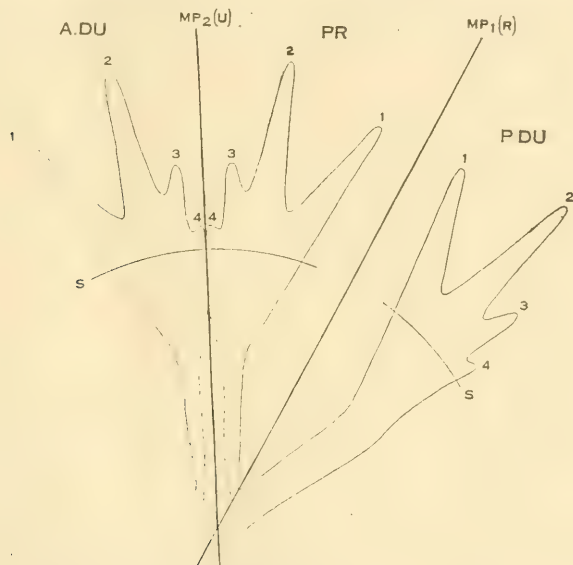
Fig. 3   Diagram showing mode of reduplication. PR, primary limb; $P.DU$,
posterior reduplicating member; $A.DU$, anterior reduplicating member; $MP_1(R)$,
primary (radial) mirror plane; $MP_2(U)$, secondary (ulnar) mirror plane; 1 to 4,
first to fourth digits, respectively. S, location of section shown in figure 4B.
Dotted lines show the outlines of limbs as they would have been had there been
no coalescence.

be mirrored in a palmar or a dorsal plane, according as the palms
or the backs of the hand face one another; when the plane is
parallel to the dorsopalmar axis, the mirroring is in a radial or
an ulnar plane, according as the radial or ulnar borders of the
limb face one another (fig. 4, A).   Intermediate planes are de-
scribed as radiodorsal, ulnopalmar, etc. (fig. 4, B).   No attempt
has been made for the present to measure accurately the angles
of mirroring.   It has been found, in agreement with Bateson, that

when there is a double reduplication, then the two mirror planes intersect at the bifurcation in a line perpendicular to the proximo-distal axes; i.e., so that with reference to the radioulnar and dorso-palmar axes the planes of reflection face one another (fig. 4). Considerable deviation from this rule has, however, been noted in certain cases, and the amphibians do not seem to follow it with the same regularity as the arthropods, according to Bateson.[10]
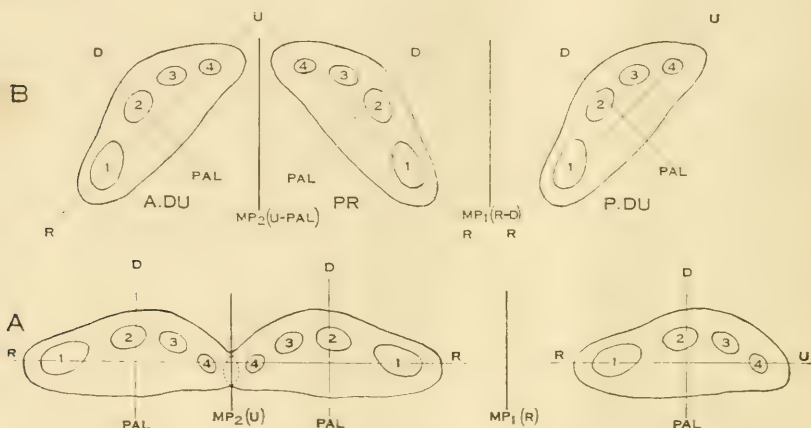
Fig. 4 Diagram of reduplication, sectional view. In A the mirror planes are radial (MP_1) and ulnar (MP_2), and a certain amount of coalescence between the primary and the anterior reduplicating members is shown, as in figure 3. In B the mirror planes are radiodorsal (MP_1) and ulno pulnar (MP_2). D, dorsal; PAL, palmar; R, radial; U, ulnar.

EXPERIMENTAL

A. Limb buds implanted in abnormal location—heterotopic transplantations

In nearly all of the experiments in this group the limb bud was implanted on the flank of the embryo at the ventral border of the myotomes between the region of the fore and hind limbs. In a few cases it was placed on the side of the head between the eye and the ear, but the grafts were absorbed in all of these except

10 Op. cit., p. 552.

one, which yielded an imperfect appendage. They need not be considered separately here, though a more extensive series of experiments of the latter type would probably yield different and more interesting results.

The limb buds transplanted to the flank of the embryo are placed in an environment similar to that of the normal fore limb, as far as relations to the body wall and muscle plates are concerned, though they lack the specific blood supply and innervation of the limb region. Consequently, a very high percentage

TABLE 1

Heterotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Posi- tive[1]	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
Hom. dd.............	19	7	3	42.8	0	00.0	4	57.1
Hom. dv.............	31	12	0	00.0	11	91.7	1	8.3
Het. dd.............	28	10	8	80.0	0	00.0	2	20.0
Het. dv.............	60	16	1(?)[2]	6.3(?)	7	43.8	8	50.0
Total.............	138	45	12	26.7	18	40.0	15	33.3
Average of percentages				32.3		33.9		33.9

[1] Excluding all cases where death occurred prematurely or where the grafted limb was resorbed or remained rudimentary. Percentages in all tables have been calculated on the basis of positive experiments.

[2] There is evidence that in this case there was an error in the orientation of the bud and that it should therefore be classed in the group het. dd.

of cases yielded only abortive limbs, and those that did develop rarely showed any functional activity.[11] There is also greater difficulty in securing good healing of wounds in the intermediate region, so that a larger proportion of the cases died early. In many of these cases there is obviously some interference with the development of the pronephric duct, which becomes blocked. The secretion which accumulates causes the formation of a cyst of considerable size, which may interfere with the development of the limb bud.

The results of the experiments are summarized in table 1.

[11] Cf. Detwiler, '19 and '20.
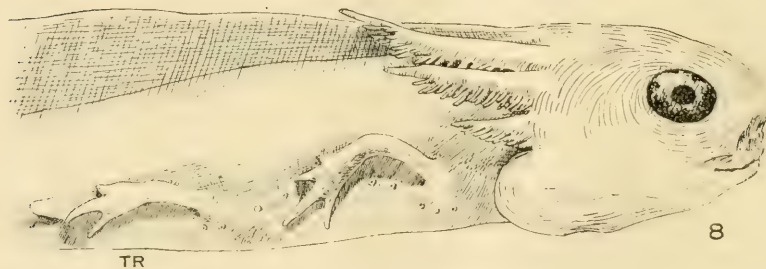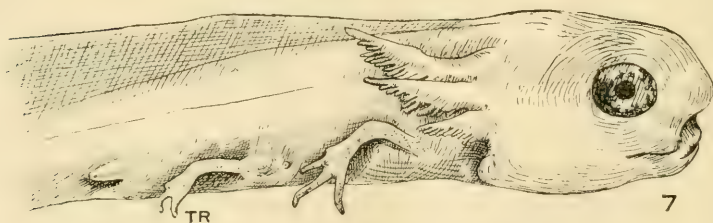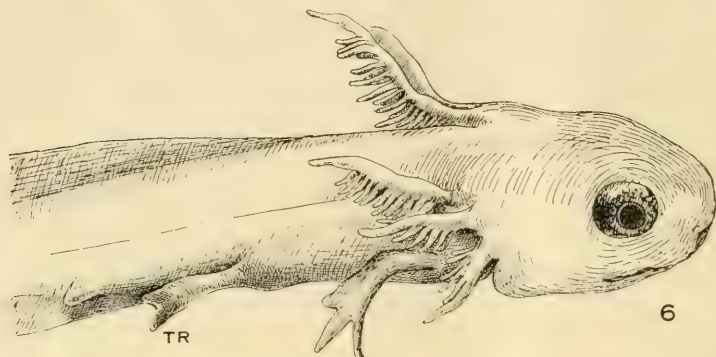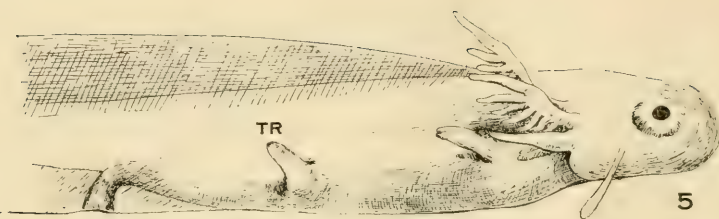
Figs. 5 to 8   Heterotopic transplantation of fore limb; right limb to right side (*hom.dd.*).   *TR*, transplanted limb.   × 10.

Fig. 5   Exp. Tr. E. 148, eight days after operation.

Fig. 6   Same, twenty days after operation.

Fig. 7   Same, twenty-eight days after operation, drawn from preserved specimen.

Fig. 8   Experiment Tr. E. 154, drawn from preserved specimen, killed twenty-two days after operation.

1. Homopleural transplantations, normal or dorsodorsal orientation. Nineteen cases were operated upon in this way (table 1). In all of the cases where observations are recorded (thirteen in number), the limbs, in the course of their development. gave evidence of their original orientation, in that they pointed posteriorly or dorsoposteriorly when they first began to grow out (fig. 5). In the three cases that gave rise to single limbs they contin-
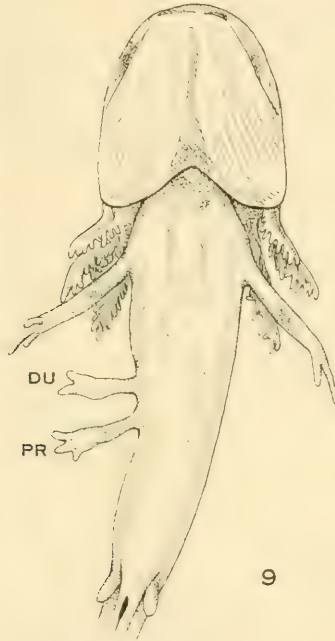
Fig. 9   Heterotopic transplantation (*hom.dd.*), showing twin limbs from one implanted bud; *PR*, primary member; *DU*, reduplicating member.   Exp. Tr. E. 182.   × 10.

ued their growth in this direction, developing almost exactly like the normal (figs. 6, 7, and 8).   Likewise in the four cases that gave rise to double appendages, the transplanted buds first began to grow in a dorsoposterior direction, and only later did the reduplicating buds appear on the anterior border of the original limb.   The original bud developed in each case into a limb of the same side, and the reduplicating buds became limbs of opposite asymmetry (fig. 9).   Histories of typical cases are given in the appendix (p. 119).
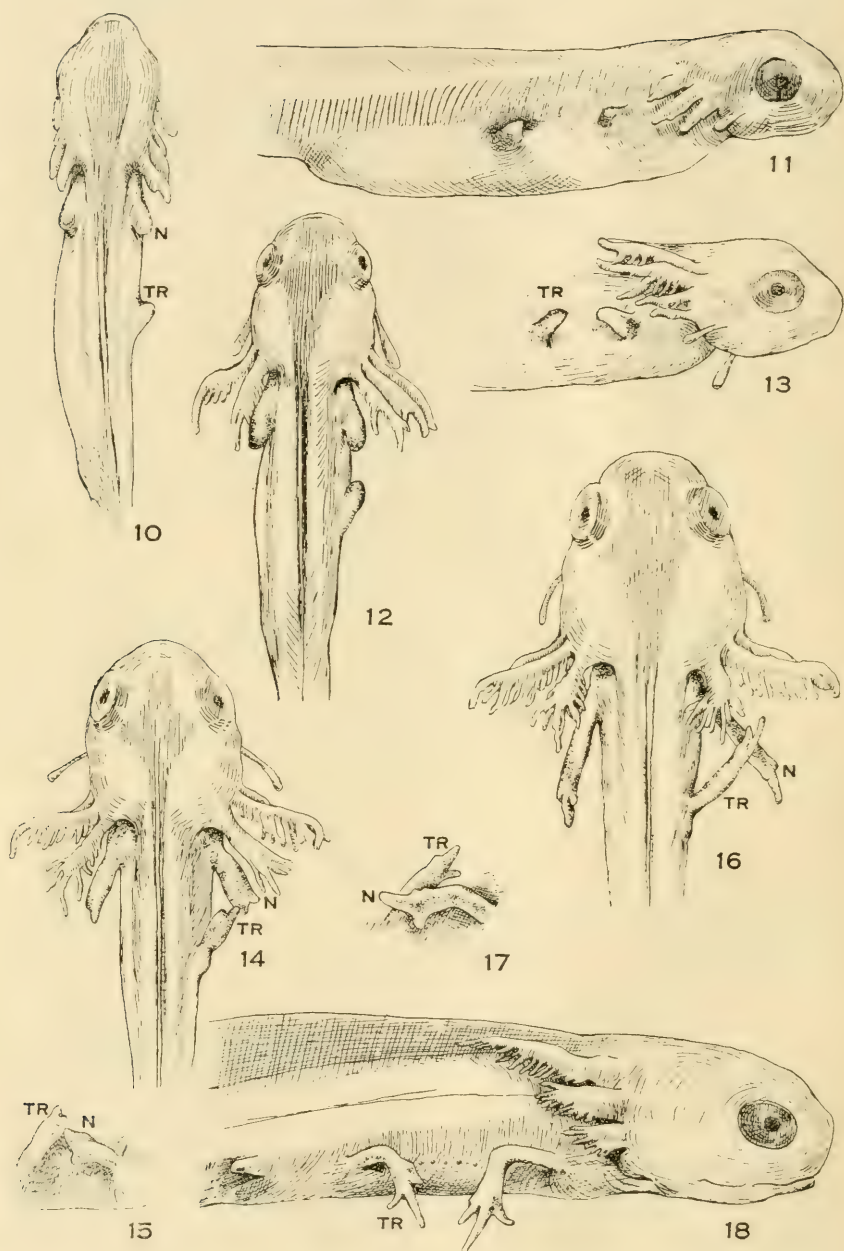
2. Homopleural transplantations, inverted or dorsoventral orienta-tion. Thirty-one experiments of this kind were made, with re-sults as shown in table 1. Of the twelve cases yielding positive results, one[12] gave rise to a pair of limbs and the others to single limbs in which the asymmetry was reversed; i.e., the right limb bud when placed upside down on the right side of the body gave rise directly to a left limb. Even in the case which showed redu-plication the primary limb of the pair became reversed. In all of the cases where limbs resulted, the initial direction of point-ing was anterior or dorsoanterior (figs. 10 and 11); i.e., nearly the opposite of normal. In four other cases this was also true. In only four cases is the direction of pointing recorded as poster-ior, and from these nothing definite was developed. All limbs which developed continued their growth in the same general direction, sometimes being directed more dorsally and sometimes more sharply anteriorly (figs. 12 to 17). They also showed the tendency to project more directly to the side than the normal limbs. The final posture assumed by these appendages varies considerably and does not seem to be dependent upon the degree of development attained by the appendage. Two cases, each having perfectly developed hands, exhibit the following extreme conditions: One[13] is practically a perfect mirror image of the nor-mal right limb both as regards form and posture (fig. 18). The

Figs. 10 to 17   Heterotopic transplantation of fore limb; right limb bud to right side inverted (*hom.dv.*), Exp. Tr. E. 219.   *N*, normal limb, right side; *TR*, transplanted limb. $\times$ 10.

Fig. 10   Dorsal view, five days after operation.

Fig. 11   Lateral view, same age.

Fig. 12   Dorsal view, eight days after operation.

Fig. 13   Lateral view, same age.

Fig. 14   Dorsal view, twelve days after operation.

Fig. 15   Lateral view of limbs only, same age.

Fig. 16   Dorsal view, sixteen days after operation.

Fig. 17   Lateral view, same age.

Fig. 18   Heterotopic transplantation; right limb to right side inverted (*hom. dv.*), Exp. Tr. E. 139; drawn from specimen preserved twenty-eight days after operation. $\times$ 10.

[12] Tr. E. 220.

[13] Tr. E. 139.

upper arm runs dorso-anteriorly and laterally. The elbow bend
is somewhat less than 90° and the fore arm and hand extend antero-
ventrally and laterally. The extensor surface of the elbow-joint
faces dorsally and slightly anteriorly and medially. The palm
of the hand faces medially, anteriorly, and slightly ventrally.

Fig. 19   Heterotopic transplantation (*hom.dv.*), Exp. Tr. E. 140; drawn from
specimen preserved twenty-eight days after operation.   *TR*, transplanted limb.
× 10.

The other case[14] has its upper arm transverse and horizontal, and
its fore arm extends ventroposteriorly at an angle of less than
45° to the horizontal axis (fig. 19). The palm looks ventrally
and anteriorly. In order to bring this limb into the position of
the former, it would have to be rotated about the axis of the
humerus 45° or more and then adducted dorsoanteriorly at the
shoulder-joint through about 30°. The difference in position
assumed by the limbs in the various cases is thus due to differ-

[14] Tr. E. 140.

ences in the amount of rotation, etc., undergone during the later stages of development.

Histories of these cases are given in the appendix (p. 120).

3. *Heteropleural transplantations, dorsodorsal orientation.* Twenty-eight experiments in this class have been made (table 1). Five of these died prematurely, and in twelve the tissue was either resorbed or failed to develop beyond the nodule stage. In one case[15] the bud developed into a stump about as long as the upper arm, but without digits. Two cases gave double limbs and eight developed into limbs which preserved their original prospective asymmetry. Two other cases may belong in this category, one in which the original orientation of the bud is recorded as uncertain[16] and another[17] in which it is recorded as dorsoventral probably by mistake.

In the development of the limb buds in this group twenty-one, in addition to the two doubtful cases just mentioned, are recorded at first as pointing in an anterodorsal direction, thus preserving their original tendency in this respect. In the eight cases in which the pointing was slight and in the five in which no definite pointing was observed the limbs were abortive or resorbed.

In the eight cases where single limbs of the side of origin developed they retained their posture, developing as nearly exact mirror images of the normal fore limb of the side to which they were transplanted (figs. 20 to 23). The elbow-joint points dorso-anteriorly, though varying somewhat, and the palm of the hand faces ventrally, medially, and anteriorly (figs. 24 and 25). Individual cases show variations similar to those observed in the previous group. It is a striking fact that the general type of development is the same here in the heteropleural non-inverted buds as in the homopleural inverted bud, which shows that both the posture and the asymmetry of the limb depend upon some reaction between the bud and its new environment. (For case histories see p. 121.)

The cases which showed reduplications, but two in number, differ considerably from one another. In the first[18] growth was slow and the resulting limb short with irregular reduplications

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[15] Tr. E. 118.   [16] Tr. E. 117.   [17] Tr. E. 113.   [18] Tr. E. 119.
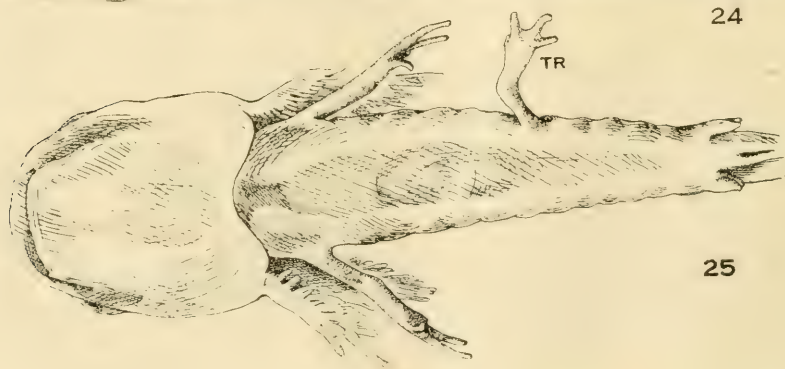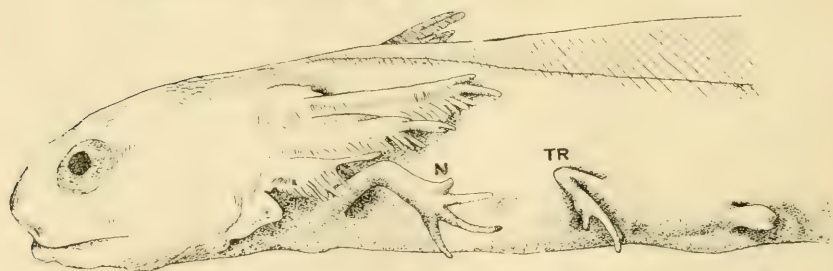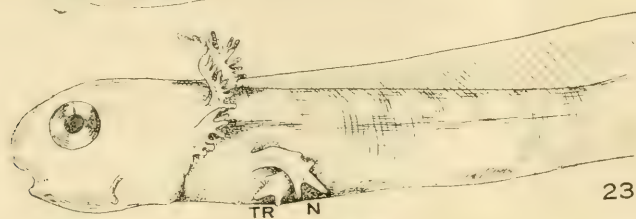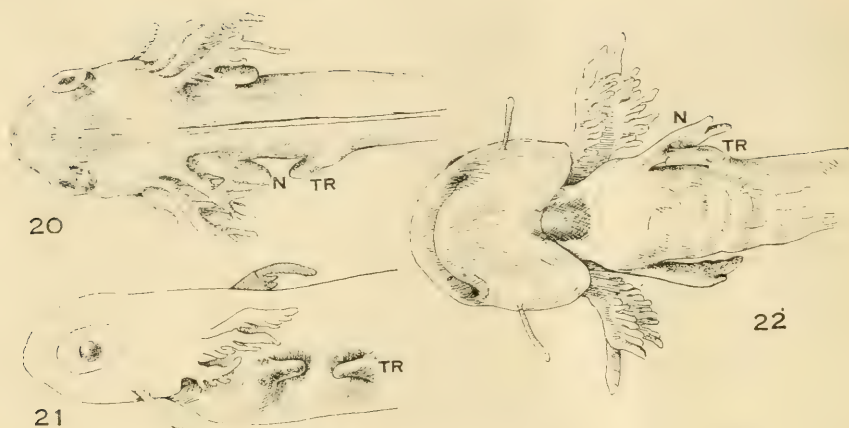
in the hand, so that right- or left-sidedness could not be determined. In the other[19] the limb developed promptly and formed a duplicate member (fig. 26), which was first seen at ten days and
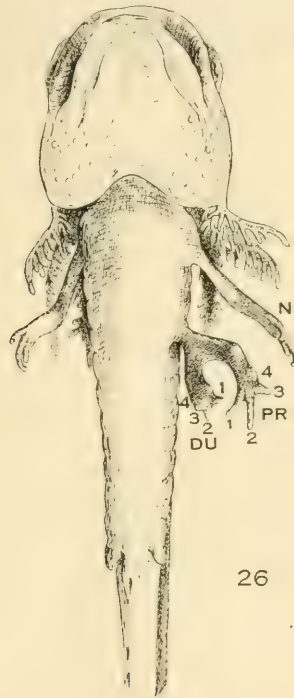
Fig. 26 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 127. Drawn from specimen preserved twenty days after operation. *PR*, primary; *DU*, reduplicating member; *1* to *4*, digits.

Figs. 20 to 23 Heterotopic transplantation of fore limb; right limb bud to left side (*het.dd.*), Exp. Tr. E. 227. *N*, normal left limb; *TR*, transplanted limb. × 10.

Figs. 20 and 21 Dorsal and lateral views, respectively, eight days after operation.

Fig. 22 Ventral view, thirteen days after operation.

Fig. 23 Lateral view, seventeen days after operation.

Figs. 24 and 25 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 107. Lateral and ventral views, respectively, of preserved specimen, twenty-six days after operation. × 10.

[19] Tr. E. 127.

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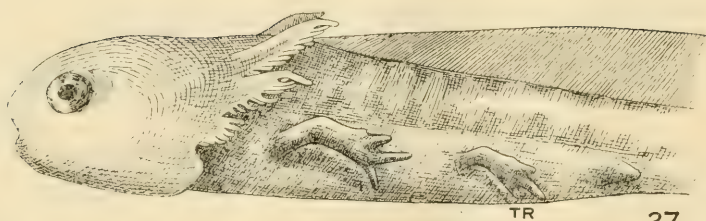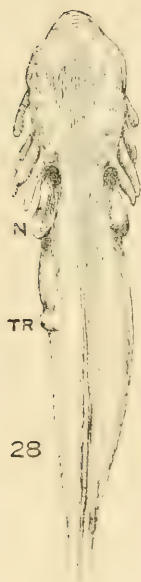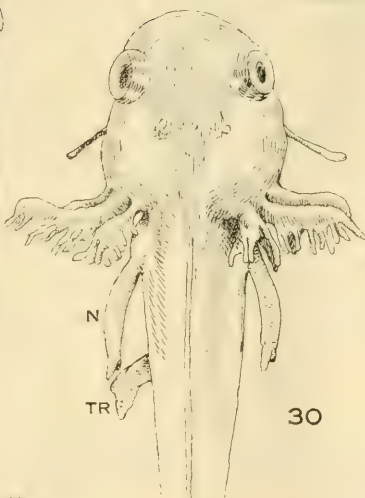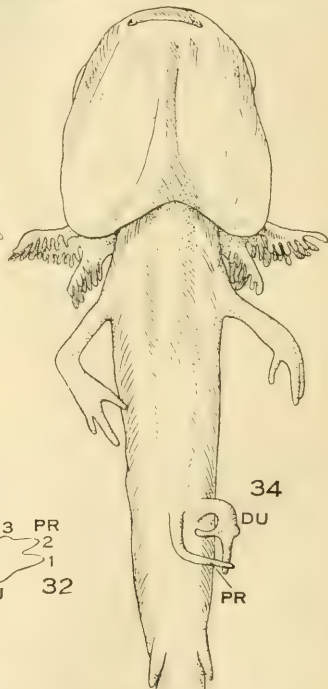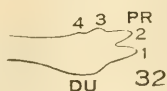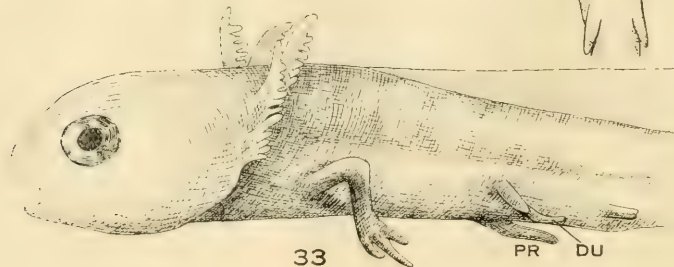
which developed ultimately into a left limb, the mirror image of the primary member, having been reversed from the original prospective asymmetry of the transplanted bud.

4. Heteropleural transplantations, dorsoventral orientation. Sixty operations were done in this series. For some unknown reason a very large proportion (twenty cases) died prematurely and sixteen of the survivors yielded only abortive limb buds, leaving only twenty-four available for consideration. Eight of these are recorded as imperfect, six produced reduplications to some degree, and nine, single limbs of reversed asymmetry. Several of the latter were somewhat defective and others showed slight reduplications. Several cases which are exceptional will be considered below.

In the cases where single limbs arose, development took place in a manner fundamentally like that of the limb buds normally oriented (*hom. dd.*). As the buds grew out, they began to point in a posterior direction, and so continuing, developed into limbs in normal posture (fig. 27). There was, however, less regularity than in the homopleural dorsodorsal group. The direction of pointing was not always dorsoposterior, as in the normal limb, but was in many cases inclined more ventrally. There are records of pointing in all of the positive experiments and in many of the negative. In only three cases is the direction recorded

Fig. 27   Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 193.   Preserved specimen killed twenty-four days after operation.   × 10.

Figs. 28 to 32   Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 217.   *N*, normal left limb; *TR*, transplanted limb; *PR*, primary member; *DU*, reduplicating member; *1* to *4*, numbers of digits.

Fig. 28   Dorsal view, five days after operation.

Fig. 29   Lateral view, five days after operation.

Fig. 30   Dorsal view, fifteen days after operation.

Fig. 31   Lateral view of limbs, fifteen days after operation.

Fig. 32   Limb showing beginning of reduplicating digits (*DU*) on ventro-anterior border (from a free-hand sketch nineteen days after operation).

Figs. 33 and 34   Heterotopic transplantation (*het.dv.*); right limb bud to left side.   Exp. Tr. E. 163.   Anomalous result.   Primary member (*PR*) defective; reduplicating member (*DU*) reversed.   Lateral and ventral aspects, respectively, drawn from specimen preserved thirty-nine days after operation.   × 10.

as dorsoanterior; one of these died early and the other two gave rise to imperfect limbs with indeterminate asymmetry.[20]

The individual cases in which limbs of opposite asymmetry developed were rather more irregular than in the preceding groups, though the best cases gave perfect reversed appendages. In addition to the ones included in the tabulation, there is one other case that probably belongs in this category. It is one in which the orientation of the bud at the time of transplantation is recorded as uncertain.[21] The limb that developed is a perfect one of reversed asymmetry in nearly the same posture as the normal limb of the side to which it was transplanted. It showed an unusual amount of motility. In one case, included in the records of this group,[22] the transplanted bud developed into a normal limb of the side from which it was taken. It is believed, however, that a mistake was made in recording the operation in this case, and that probably in reality the orientation of the limb was not inverted. The direction of pointing, as observed on the third and fifth days after the operation when the limb bud is recorded as pointing anteriorly, is evidence, though not absolutely conclusive, that an error has been made. If this interpretation is correct, the case would not be exceptional, but would accord with the eight cases described in the previous section.

In the eight cases in which reduplications occurred, the early stages of development were like the normal (figs. 28 and 29), the reduplicating buds not being noted until at least twelve days after the operation. Three individuals showed distinctly that the primary limb was of reversed asymmetry. In one case it was so imperfect that it could not be determined to which side it belonged, but the reduplicating limb was sufficiently developed to show that it was of the same side as the bud was originally, indicating that the original member was in all probability reversed. Another case[23] gave a limb with nearly symmetrical reduplication in the hand without anything to indicate which member was primary (figs. 31 and 32). Two long radial digits are present in the middle and two short ulnar digits on each side. Still another case[24] gave a very peculiar result. The primary

[20] Tr. E. 108 and 203.　　[21] Tr. E. 109.　　[22] Tr. E. 113.　　[23] Tr. E. 217.
[24] Tr. E. 163.

limb bud developed into a long almost filiform structure, with-
out digits, that grew posteriorly on the ventral side of the body
not far from the midline. Twenty days after the operation a sec-
ond bud was noticed dorsal to the original, and this developed
into a somewhat peculiarly placed limb. The upper arm runs
transversely and the palm of the hand faces dorsomedially (figs.
33 and 34). This limb is clearly a left; i.e., its original prospective
asymmetry has been reversed. It therefore constitutes an ex-
ception to the rules, not only because of the position of the
hand, but also because of its particular asymmetry; for the original
(filiform) member should have been reversed (a left), and the
second one reversed back again to the original asymmetry. How-
ever, the fact that the latter developed at such a considerable dis-
tance from the original member, might be regarded as indicating
that it was beyond its sphere of influence, perhaps having been
split apart from it at an early stage, and that it remained there-
fore as of the same side. Several cases of regeneration after
extirpation of half buds and of transplantation of half buds gave
analogous results (fig. 132).[25]

5. *The shoulder-girdle in heterotopic transplantations.* The
limb-girdle in the hetrotopic transplantations is developed in
more or less reduced condition, as was first shown by Braus ('09)
in the anurans. Detwiler ('18) has studied this question in
Amblystoma, and has found that the degree of development of
the girdle is dependent upon the size of the graft and the region
from which it is taken, the scapula and suprascapula being local-
ized in the tissue dorsal to the normal limb bud and the coracoid
in that ventral to it.[26] The form of the reduced girdle derived

[25] Cf. Harrison, '18, p. 441 (Exp. Rem. E. 17 and H. R. E. 10), and page 135
of the present paper (Exp. H. R. E. 20).

[26] It is a curious fact that in the embryo the limb-girdle has undoubtedly
the character of a mosaic, without totipotence of its parts, while in the adult
Triton, according to Tornier ('06), Fritsch ('11), and Kurz ('12), a small portion
of the shoulder-girdle can regenerate the whole, including the fore limb. Ac-
cording to the two last-named investigators, even if the whole girdle is removed,
it will be regenerated together with the free appendage. Kurz has found that
this holds for both shoulder and pelvic girdles but that removal of the sacral
portion of the vertebral column prevents regeneration. In the anurans, accord-
ing to Braus ('06), there is considerable variation in the regenerative powers of
the limbs in early stages.

from the usual round disc (limb bud) is roughly triangular, as shown in the figure of Detwiler's model (his figure 28), with a ventral process projecting anteriorly, to be identified as a rudimentary coracoid, and a dorsal process, which includes the rudiment of the scapula. In the normally oriented grafts (homopleural dorsodorsal) these processes point anteriorly, with a single process projecting posteriorly slightly behind the glenoid cavity. This shows clearly in two cases.[27] The question now arises whether the girdle follows the rules governing the asymmetry of the free limbs. The results, in the main indicate that such is the case, though the girdle developed is often so small and rudimentary, that it is not possible to determine to which side it belongs. In the inverted homopleural grafts, which give rise to reversed limbs, the girdle also seems to be reversed. This is true in four cases out of the five examined in serial sections.[28]

Among the heteropleural dorsodorsal transplantations, five cases have been examined in sections. In two of them[29] with well-developed glenoid cavity, the girdle cartilage is mostly ventral and posterior to the joint. This probably represents a coracoid with asymmetry corresponding to that of the free limb. One case,[30] with the cartilage projecting both anteriorly and posteriorly from the cavity, gives no evidence as to the side to which it belongs. One is too rudimentary,[31] and one seems to have had its asymmetry reversed,[32] though the limb is not reversed. In the two dorsoventral heteropleural transplants which have been studied in sections, the side to which the girdle belongs cannot be determined. Other cases from among the earlier experiments, where in most instances the size of the transplanted bud was small, are inconclusive. On the whole, the cases where the asymmetry can be determined with any degree of certainty seem to follow the rules. Only a single case thus far examined is clearly exceptional.

[27] Tr. E. 148 and 154.
[28] Tr. E. 135, 136, 139, and 140.
[29] Tr. E. 124 and 169.
[30] Tr. E. 120.
[31] Tr. E. 127.
[32] Tr. E. 107.

6. Summary of the results of heterotopic transplantations. A survey of all the experiments in this group brings out the following facts:

Implanted in dorsodorsal orientation, a limb bud gives rise to an appendage of its original prospective asymmetry, whether placed on the same or opposite side of the body. Such appendages have a normal posture when placed on the same side of the body from which they were taken, but when placed on the opposite side they mirror approximately the limb of that side, though they often become rotated to quite different postures. Implanted in inverted (dorsoventral) position, a limb bud gives rise to an appendage of reversed asymmetry whether placed on the same or opposite side of the body. When placed on the same side, such appendages mirror the normal limb of that side, but when grafted on the opposite side, they assume a posture approximately identical with that of the limb of that side.

Limbs implanted in any of the four positions here studied may produce reduplications. As far as it has been possible to determine, the primary limb of the pair is then of the same side as a single limb would be according to the foregoing rules. The reduplicating limb has been found to be, with a single exception, the mirror image of the first.

Limbs that are grafted in abnormal location have at best very incomplete function and are often apparently entirely immobile. They usually do not become so large as those that are implanted in normal location, and they show defects and evidences of atrophy much more frequently.

B. *Limb buds implanted in natural location—orthotopic transplantation*

In these experiments the limb bud of the host was first removed and then either put back in place, or else a bud from another embryo was grafted into the wound. In all of the earlier cases the wound bed was not cleaned after removal of the bud, so that some cells from the host were left to mingle with the tissues of the transplanted limb rudiment. The later experiments, with

but few exceptions, were carried out under precautions necessary
and sufficient to preclude contamination of this kind: the extir-
pated area was three and a half somites in diameter, and the bed
of the wound was carefully scraped after removal of the bud.[33]
The results were somewhat different (proportionately) in the

TABLE 2

Orthotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Posi-tive	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent
A. Wound bed cleaned and wound not less than $3\frac{1}{2}$ somites								
Hom. dd................	9	9	9	100.0	0	00.0	0	00.0
Hom. dv................	61	38	10[2]	26.3	1	2.6	27[1]	71.1
Het. dd................	49	31	1	3.2	5[3]	16.1	25	80.6
Het. dv................	26	16	0	00.0	15	93.8	1	6.3
Total................	145	94	20	21.3	21	22.3	53	56.4
Average of percentages.				31.6		28.8		39.6
B. Wound bed not cleaned								
Hom. dd................	0	0	0		0		0	
Hom. dv................	37	20	19[4]	95.0	0	00.0	1	5.0
Het. dd................	17	13	2	15.4	3	23.1	8	61.5
Het. dv................	21	15	0	00.0	8	53.3	7	46.7
Total................	75	48	21	43.8	11	22.9	16	33.3

[1] Including three cases in which the primary bud righted itself by rotation and
the duplicate is disharmonic.

[2] Limbs which became normal by rotation, including one case (I. E. 101) of
hyperdactyly.

[3] Normal by resorption of original member of pair.

[4] One case included in which the posture of the limb was abnormal.

two classes of experiments and have been summarized separately
in table 2 (A and B).   The differences will be taken up in connec-
tion with the consideration of each of the subgroups.

7. *Homopleural transplantations, dorsodorsal orientation.*   This
is in reality merely a control experiment and is a test of the effect

[33] Harrison, '15 and '18, p. 422.

of the operation as such on the development of the limb. A fore limb bud is carefully excised and either replaced in the same wound or else engrafted in normal position in another embryo from which the limb bud had been previously removed.

Only nine individuals were operated upon, in all of which the wounds were carefully cleaned. Normal limbs developed in all cases, though they were slightly retarded in the earlier stages of development in comparison with the unoperated limb of the opposite side. In six of the cases the pronephros was removed and in the other three it was left in. No difference was noted between the two sets. It may be safely concluded that the effect of the operation itself upon normal development is practically negligible.

8. *Homopleural transplantations, dorsoventral orientation.* In some of the cases of this series, as in the last, the limb bud was simply lifted and replaced after rotation through 180°. In the others the wound bed was first prepared in one embryo and the bud taken from another. The latter method is preferable and it was employed in all the later experiments.

The total number of experiments is one hundred and four, of which sixty-one were with cleaned wounds of proper size. The latter will be considered first, since the conditions of experimentation are more definitely known and there can be no doubt that the limbs were derived exclusively from the transplanted tissue.

Leaving out of consideration the twenty-three cases which died prematurely or gave rise merely to abortive or rudimentary limbs, there are thirty-eight cases which yielded positive results, as recorded in table 2A. The single limbs are in the minority and are of two kinds, reversed and non-reversed. The most remarkable case[34] (history on p. 124), which really gives the clue to the interpretation of the experiments of this group, is the one in which a limb of reversed asymmetry developed, a right limb on the left side, perfectly normal in form, function, and posture, as far as the last is possible on the wrong side of the body (figs. 35 to 41). The shoulder-girdle of this limb is also reversed and

[34] I. E. 64.
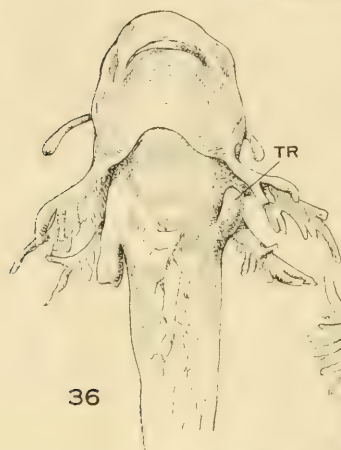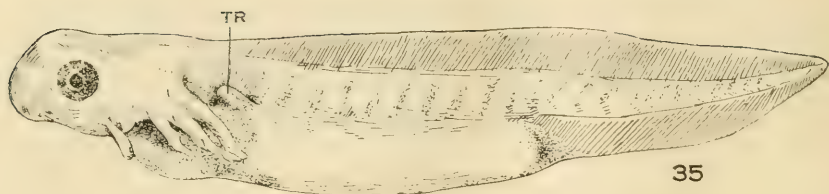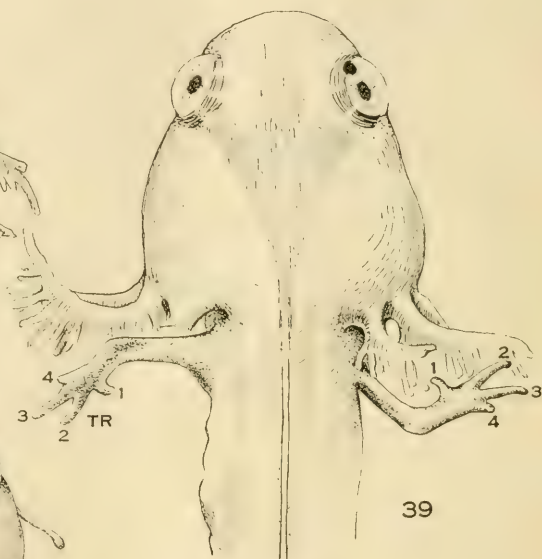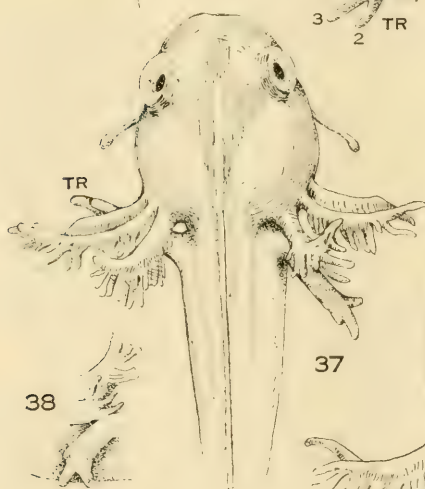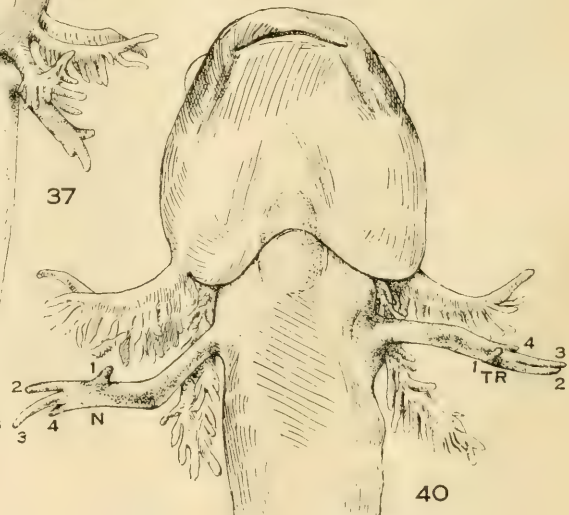
is quite separate from the rudimentary girdle developed from the tissues of the host (p. 59).

The ten cases in which normal non-reversed limbs developed are clearly contrary to the rule (p. 4). The records of these cases show that the end result is reached by a process of rotation at the shoulder-joint during development (figs. 56 to 58). They will be considered below (p. 40). The reduplicated limbs, of which there were twenty-seven, fall, like the single, into two groups. In the first the original bud developed into a limb of reversed asymmetry, while in the second it is not reversed.

Observations upon the earlier stages of the operated limbs show that in those cases which give reduplications, as well as in the case of the simple limb with reversed asymmetry (figs. 35, 42, 43, 49, 51, and 52), the original direction of pointing is either dorsal, anterior, or dorsoanterior, and more sharply lateral than normal. Likewise in the case of those that develop into single non-reversed limbs, the first pointing is more sharply lateral than normal, and also more dorsal, though only two[35] are recorded as pointing slightly anteriorly from the dorsal direction. This shows that the original tendencies of growth, immanent in the bud at the time of transplantation, are by no means inactive when it is in its new position. One or the other of two conse- quences of this growth tendency now ensues, indicating a sort of antagonistic reaction between the organization of the transplanted rudiment and that of the surrounding parts. The limb either continues to grow in an anterior or anterodorsal direction, in

Figs. 35 to 41 Orthotopic transplantation; left limb to left side inverted (*hom.dv.*), resulting in a normal right limb on the operated side. Exp. I. E. 64. *TR*, transplanted limb. × 10.

Fig. 35 Lateral view, five days after operation.

Fig. 36 Ventral view, ten days after operation.

Fig. 37 Dorsal view, sixteen days after operation; transplanted limb covered by gills.

Fig. 38 Lateral view of limb, sixteen days after operation.

Fig. 39 Dorsal view, twenty-three days after operation.

Fig. 40 Ventral view, twenty-three days after operation.

Fig. 41 Lateral view, twenty-three days after operation.

[35] I. E. 49 and 94.
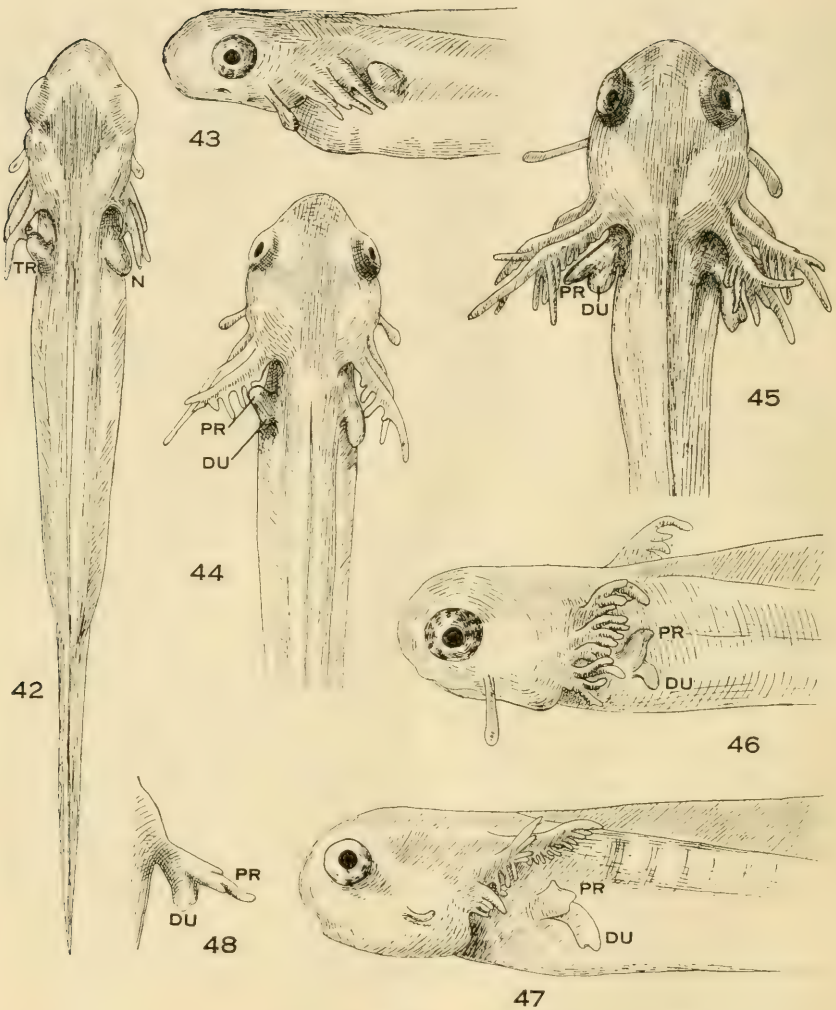
Figs. 42 to 48   Orthotopic transplantation; left limb bud to left side inverted (*hom.dv.*), resulting in duplicate limbs.   Exp. I. E. 60.   *N*, normal right limb bud; *TR*, transplanted bud; *PR*, primary member; *DU*, reduplicating member.   × 10.

    Fig. 42   Dorsal view, five days after operation.
    Fig. 43   Lateral view, five days after operation.
    Fig. 44   Dorsal view, seven days after operation.
    Fig. 45   Dorsal view, twelve days after operation.
    Fig. 46   Lateral view, twelve days after operation.
    Fig. 47   Lateral view of specimen preserved eighteen days after operation.
    Fig. 48   Ventral view of limb, preserved specimen.

which case its asymmetry is reversed (figs. 36 to 39), just as in the corresponding class of heterotopic transplantations, or it gradually rotates towards its normal position while retaining its original prospective asymmetry (figs. 56 to 59). In the former alternative duplicate limbs are nearly always formed. Only in the one case, referred to above, did a perfect single limb arise. In the other alternative single limbs usually arise, though some of the cases of reduplication certainly belong to this group.

In the duplicities belonging to the first group the original limb bud continues to grow in an anterior direction and ultimately becomes a reversed limb. After a time a reduplicating bud appears on the posterior border of the original bud (fig. 44) and in the clearer cases grows into a homopleural limb in approximately normal posture (figs. 45 to 48). The original bud becomes a reversed limb which, together with the reduplicating member, may form an almost symmetrical complex.

Twenty-four of the thirty-one[36] cases of reduplicated limbs are probably of this type. Fifteen are certainly so,[37] and in three others[38] that are very similar all that is lacking to place them unequivocally in this group is a definite observation as to which bud was the primary one; six more cases[39] may also be interpreted in the same manner, though they are not sufficiently clear to insure that this is the only possible interpretation.

The degree of reduplication varies here, as in the other groups of experiments, from the condition where almost the whole arm is involved to that in which the hand is only partly double. In three cases[40] the anterior bud was much reduced (p. 49), the posterior bud becoming a somewhat irregular homopleural limb. In eleven cases there is only one reduplicating appendage, which is always posterior to the primary (figs. 43 to 48), while in the remaining twelve[41] there are evidences of further doubling, usu-

[36] Four cases are considered here which are not included in the tabulation on account of the fact that the wound was only 3 somites in diameter (I. E. 39, 41, 44 and 45).

[37] I. E. 48, 60, 62, 63, 66, 72, 74, 75, 81, 85, 87, 89, 91, 92, and 96.

[38] I. E. 44, 45, and 52.

[39] I. E. 39, 68, 70, 93, 100, 102.

[40] I. E. 92, 93, 100.

[41] I. E. 39, 45, 62, 63, 66, 72, 75, 81, 85, 87, 91, and 93.
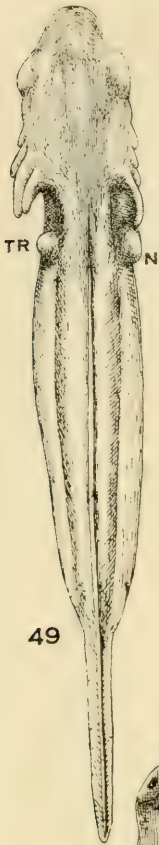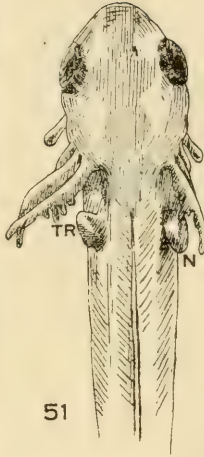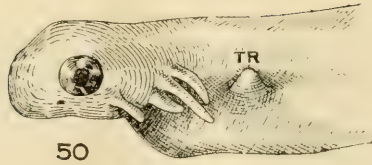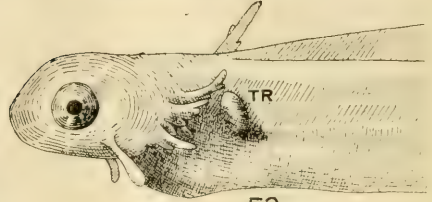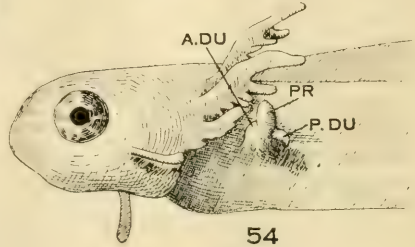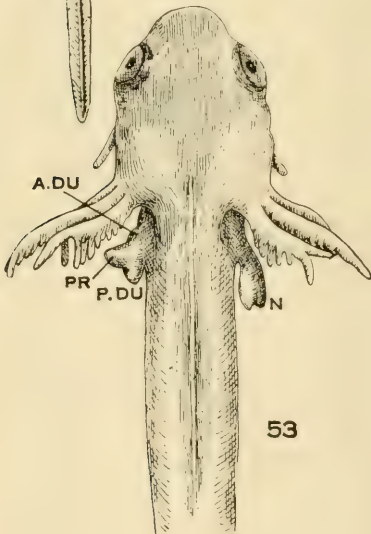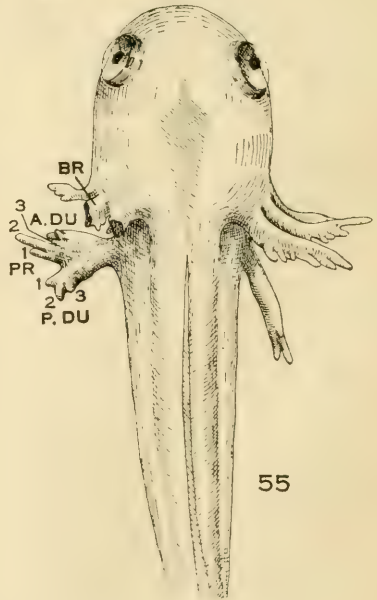
ally on the anterior side of the original (figs. 49 to 55 and 61).
When the latter condition arises and the anterior reduplicating
member is sufficiently developed, it is seen that it, too, is mirrored
from the original member and is homopleural (fig. 55). In one
case there are three complete hands, one of which has two of its
digits doubled.[42] The plane from which the posterior redupli-
cating member is mirrored in the final form of the limb varies
from radial to dorsal (figs. 3 and 4) and is usually intermediate
between these two extremes (p. 13). Nineteen cases follow
this rule, three are indeterminate and there is only one positive
exceptional case, in which the mirror plane is ulnodorsal.[43] When
there is also an anterior reduplicating member, it is generally
mirrored from a plane 180° around the limb axis from the first;
i.e., ulnar, ulnopalmar, or palmar.

The reduplications belonging to the second group are more
restricted and less certain of diagnosis. The limb bud retains its
original prospective asymmetry, reaching an approximately nor-
mal position by rotation, and reduplication is much less extensive,
involving in most cases the digits only (fig. 62). Three cases[44]
almost certainly belong to this group, and there may be two
others.[45]

Of the two remaining cases of reduplication, one died too
young; in the second[46] the supernumerary limb was of the same
side as the primary and was quite distinct from it. This is a
very unusual condition, but the transplanted bud in this case was

Figs. 49 to 55   Orthotopic transplantation; left limb bud to left side inverted
(*hom.dv.*), resulting in limb with two reduplicating members.   Exp. I. E. 63.
N, normal right limb bud; *TR*, transplanted left bud; *PR*, primary limb; *A.DU*,
anterior, and *P. DU*, posterior reduplicating members.   × 10.
Fig. 49   Dorsal view, four days after operation.
Fig. 50   Lateral view, four days after operation.
Fig. 51   Dorsal view, seven days after operation.
Fig. 52   Lateral view, seven days after operation.
Fig. 53   Dorsal view, ten days after operation.
Fig. 54   Lateral view, ten days after operation.
Fig. 55   Dorsal view of specimen preserved seventeen days after operation.
Gills (*BR*) removed to show limb.   *1* to *3*, numbers of digits.

[42] I. E. 87.   [43] I. E. 72.   [44] I. E. 86, 88, 90.   [45] I. E. 41, 59.   [46] I. E. 38.

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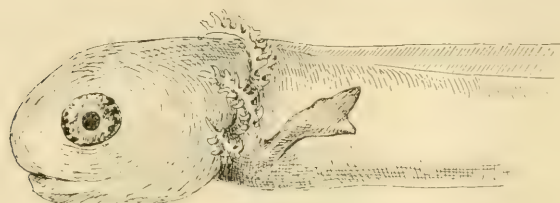
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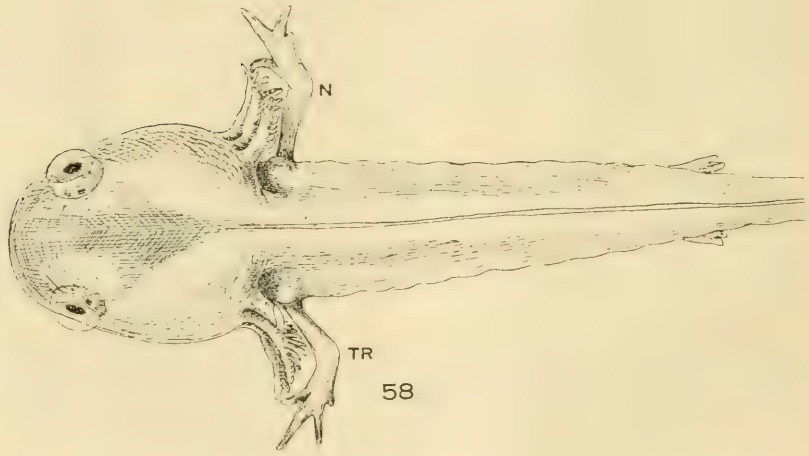
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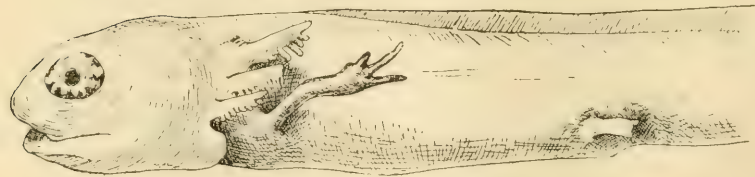
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larger than usual (four somites in diameter), and it is possible that the reduplicating bud, growing from near its anterior border, was uninfluenced by the primary limb and hence was not mirrored.

There remain for consideration those cases in which a single non-reversed limb developed. As in the other cases, the limb bud in these showed at first the consequences of abnormal orientation. When first observed it pointed more sharply laterally and more dorsally (less posteriorly) than normal. Two even pointed dorsally and slightly anteriorly. In the course of development the limb gradually changed its posture and ultimately came to a perfectly normal posture by a process of rotation at the shoulder-joint (figs. 56 to 59). Ten such cases were obtained,[47] though in several of them[48] it is possible that reversal may have been brought about by early reduplication and suppression of the original bud, as described in the next section (p. 49). In one of these[49] a supernumerary radial digit was present, but this is to be regarded as a case of hyperdactyly rather than one of mirrored reduplication. In one case[50] the limb which originally developed showed irregularities in the digits. The arm was then amputated above the elbow, and the appendage which regenerated was in every respect normal. This case is of considerable interest in showing that the abnormal condition which produces reduplication is not necessarily stamped upon the whole structure, but may be due to some local mechanical disturbance.

In reviewing this group of experiments, it is clear that the first two results, i.e., single reversed limbs and most of the reduplications, come under the same scheme. There is a primary reversal of asymmetry, without reduplication in the first case and accom-

Figs. 56 to 59 Orthotopic transplantation; left limb bud inverted (*hom.dv.*). Limb reaches normal posture by rotation. Exp. I. E. 49. *N*, normal (right) limb; *TR*, transplanted (left) limb.

Fig. 56 Eleven days after operation.
Fig. 57 Twenty-one days after operation.
Fig. 58 Thirty-eight days after operation.
Fig. 59 Preserved specimen, killed at thirty-nine days.

[47] I. E. 49, 55, 69, 71, 73, 77, 84, 94, 99, and 101.   [49] I. E. 101.
[48] For instance, I. E. 77.   [50] I. E. 71.

panied by reduplication in the second. In the latter the second-
ary bud, being the mirror image of the other, is again reversed
back to the original prospective asymmetry of the transplanted
bud. This then occupies an approximately normal position and
may function to a considerable extent as a normal limb, though
impeded by the connection with its mate. These two results are
directly comparable to those of the heterotopic transplantations
of the corresponding class (fig. 2).

The third result, in which normal homopleural limbs develop,
reaching their normal position gradually by rotation, is funda-
mentally different. Here no reversal occurs; the limb bud begins
its development as a self-differentiating system, but later, under
the stress of the changed relation to its environment, it comes
again into normal posture.

What determines whether the limb bud shall reverse its asym-
metry or rotate back to its normal posture? The earlier experi-
ments of this series afforded no satisfactory answer to this ques-
tion. It was certainly not due to the size of the wound, the
mode of preparation of the wound, the presence or absence of
the pronephros, or the age of the embryo. What seemed most
likely was that there were minor accidental differences in the
amount of rotation to which the limb bud was subjected at the
time of operation. It was conceivable, for instance, that if the
disc were rotated anteriorly around the dorsal semicircumference
of the wound a little less than 180° (fig. 60), the reversing effect
of its organic environment might be lessened and the rotation
back to normal position facilitated; in this case a normal non-
reversed limb would result. If, on the other hand, the grafted
bud were rotated 180° or slightly more, the reversing effect might
be at a maximum and rotation most impeded, in which case a
heteropleural limb or twin limbs would arise.

Experiments made in the spring of 1917 had for their main
purpose the testing of this hypothesis. Operations were done
in pairs; in one case the limb disc was rotated about the dorsal
circumference in a posteroanterior direction slightly more than
180° and in the other slightly less, extremes being probably not
more than 190° and 170°, respectively (see histories on pp. 125–6).

The results are not altogether conclusive, though they point to the correctness of the hypothesis. Twenty-three operations were done, of which seventeen yielded positive results. Ten cases harmonize with the hypothesis; four are doubtful, and three are contrary to it (figs. 61 and 62). When the difficulty of exactly estimating the degree of rotation is considered, many apparent exceptions must be expected, and a far greater number of experiments would be necessary to eliminate statistically the effect of this uncertainty. As a matter of fact, the records of the cases classed as surely exceptional give evidence that the amount
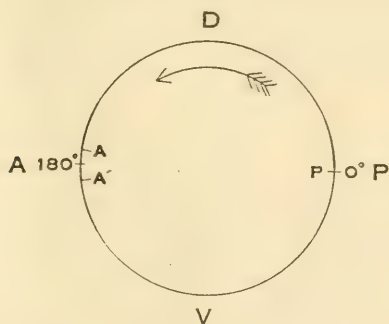
Fig. 60 Diagram showing difference in amount of rotation in two sets of experiments. The circle represents the left limb bud and the arrow the direction of rotation. A, D, P, V, the direction of the cardinal points of the embryonic body, anterior, dorsal, posterior, and ventral, respectively.

of rotation at the time of operation was probably not correctly estimated, for the first direction of pointing (p. 11) in all of these cases is not according to expectation.

The thirty-seven cases in which the wound bed was not entirely cleaned of mesoderm may now be considered. These show a marked contrast to those with cleaned wounds, inasmuch as there are very few reduplications and a very large proportion of normal non-reversed limbs. Thus out of twenty cases which yielded positive results eighteen or 90 per cent are normal, as compared with 23.8 per cent (ten cases) in the clean-wound class. Only one case (5 per cent) had a reduplicated limb, as compared with thirty-one duplicities (73.8 per cent) in the clean-wound class.
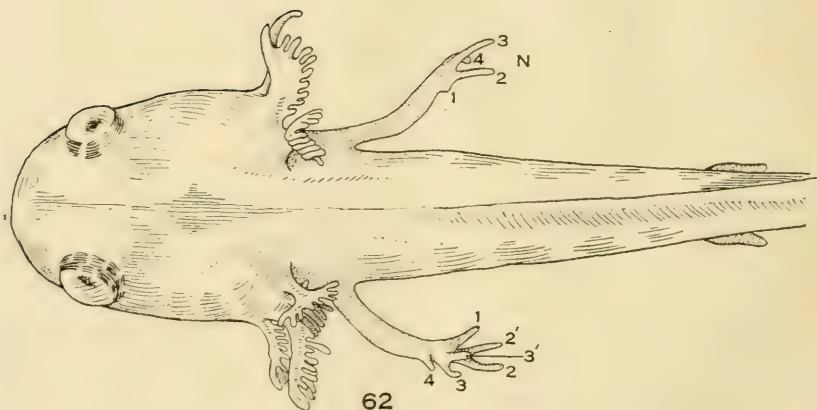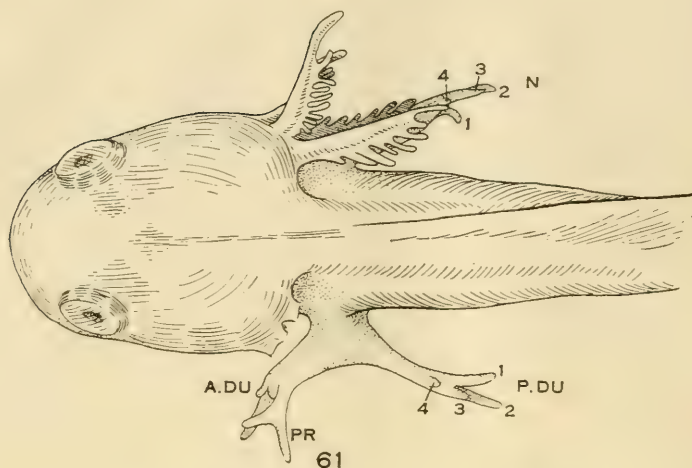
Fig. 61 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly more than 180° from its normal position, *i.e.* P to A′ in fig. 60. Exp. I. E. 85. Primary member (*PR*) is a right; posterior reduplicating member (*P.DU*) is a nearly normal left; anterior reduplicating member (*A.DU*) partly coalesced with primary.

Fig. 62 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly less than 180° (*i.e.* P to A in fig. 60). Exp. I. E. 86. The transplanted limb is primarily a left, having reached its normal posture by rotation; second (*2′*) and third (*3′*) digits, reduplicated. × 10.

Since these differences can scarcely be accounted for on the ground of different degree of rotation of the limb buds at operation (p. 40), it would seem that the few mesoderm cells remaining in the wound bed must have exerted some influence upon the developing limb. This does not mean that the limbs which do develop in such cases arise solely by a process of regeneration from the host. In fact, the rate of development, which is only slightly retarded below the normal, precludes such an interpretation. What probably does take place is an intermingling of cells from the host and the graft, with the result that the former, acting in the same sense as the environment with which they are in harmonic relation, counteract the tendency of the inverted elements to reverse their asymmetry. This was, however not shown to the same degree in the corresponding experiments with superposed limbs (p. 65).

9. *Heteropleural transplantations, dorsodorsal orientation.* Forty-nine cases were operated upon in this way and thirty-one lived long enough to yield definite results (table 2). By far the largest number of these (twenty-five) developed reduplications of one kind or other. Five cases gave rise to limbs with reversed asymmetry, i.e., to limbs which developed to fit their new surroundings, though one of these was considerably underdeveloped. One yielded a somewhat imperfect non-reversed limb and four were rudimentary. These results seem altogether divergent from the corresponding heterotopic transplantations. An examination of them shows, however, that fundamentally they accord with the latter, complete agreement being modified, by a second factor, which may suppress the original bud in favor of the reduplicating member. The normal environment of the transplanted bud and the concomitant normal functioning seem to facilitate this transformation. Moreover, there is no hard and fast line between the different results just enumerated, and the individual cases may be taken as forming a series, beginning with the single non-reversed and ending with the single reversed limb. The reduplications are intermediate. They will be considered in this order.
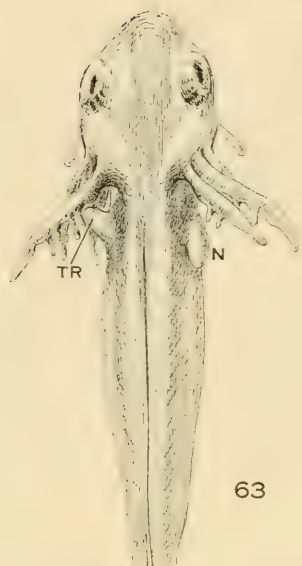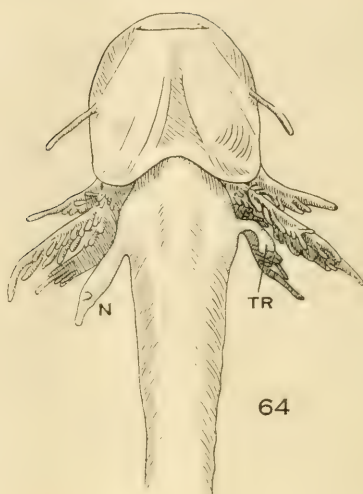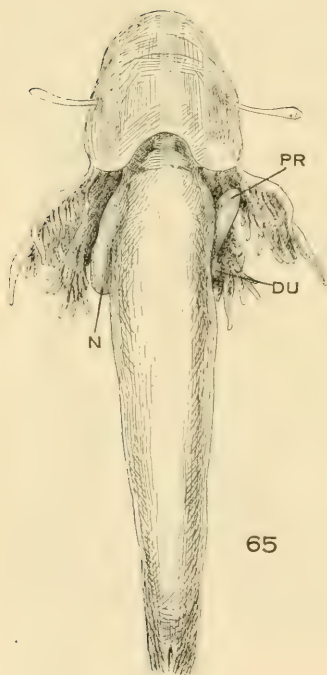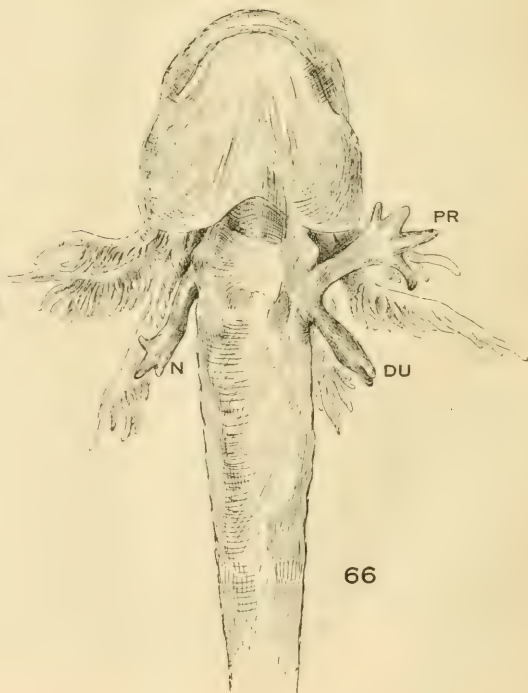
The history of the case in which a limb of original prospective asymmetry developed[51] is given on page 126. In this individual the limb bud, as it began to grow, pointed anteriorly (fig. 63), and continued to grow in that direction. Though it remained small and imperfect (fig. 64), it is clearly a right limb on the left side (not reversed).

The cases which formed reduplications began their development in the same manner. The first direction of pointing is recorded as anterior in nine cases, anterodorsal in eight, and anterolateral in five. Three are described as pointing dorsally and one laterally. Thus these limbs all show in greater or less degree the initial effect of their original growth tendency. Growth of the bud continues then for some days in a general anterior direction, but sooner or later a reduplicating bud appears, usually at the posterior border of the original bud, and this grows in most cases into an appendage equal to or exceeding the original in size. If the reduplicating bud does not appear until late, then the original one may attain considerable size and remain, for some time at least, the principal member (figs. 65 and 66). If it appears earlier, but not until the original bud has a good start, then the two members may remain of almost equal size (figs. 67 to 71). In other cases, where the reduplicating bud begins to grow early, it soon gains the upper hand, and the original may be reduced to an atrophic or rudimentary limb (figs. 72 to 74). This condition leads over to the single reversed appendage in which the original bud is reduced to a spur or nodule

Figs. 63 and 64 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 87. Resulting limb, though defective, is reversed. *N*, normal right limb; *TR*, transplanted limb. × 10.

Fig. 63 Dorsal view, seven days after operation.

Fig. 64 Ventral view of specimen preserved sixteen days after operation.

Figs. 65 and 66 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 96. Resulting limb reduplicated.

Fig. 65 Ventral view, ten days after operation; primary limb (*PR*) points into gills; reduplicating bud (*DU*), just appearing. × 10.

Fig. 66 Ventral view, nineteen days after operation; primary member (*PR*) shows evidence of reduplication of hand; reduplicating member (*DU*) is in approximately normal position. × 10.

[51] R. E. 87.
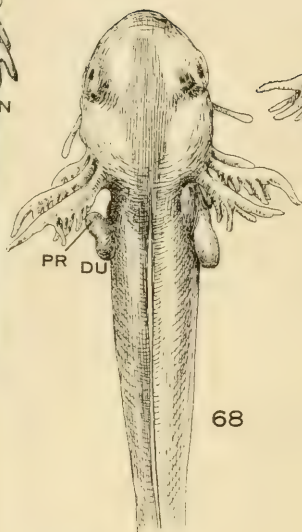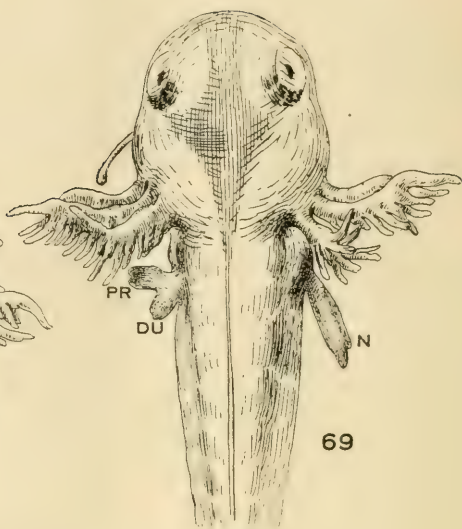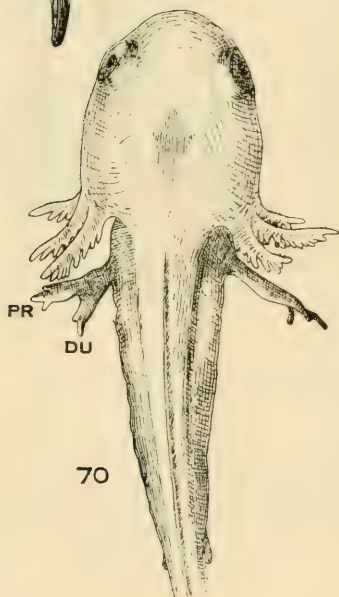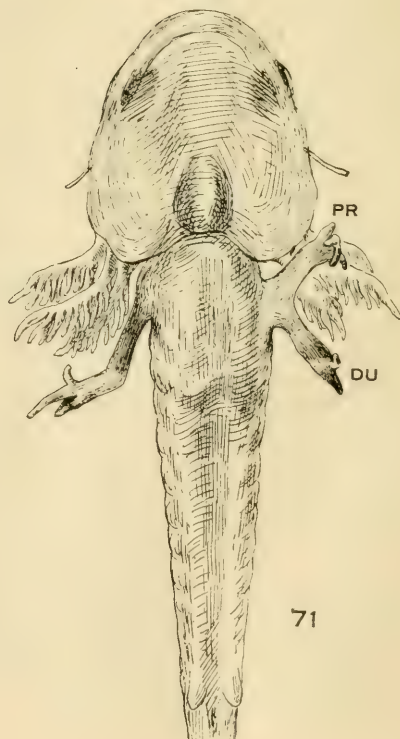
(p. 49).   The reduplicating limb is, of course, mirrored from the original and hence corresponds to the side of the body on which it is grafted.

Being placed in the position of the normal limb, the reduplication is favorably situated with regard to blood and nerve supply, and it is probably on this account that so many of them develop into functional appendages.   In this respect the experiments of this group differ considerably from the preceding (homopleural inverted), where there is a greater tendency for the original member to retain its predominant condition.   Otherwise the course of development in the two groups is strikingly alike.

For the study of the details of reduplication, nineteen cases are available, including one case with small wound not considered in the table.   Seven others were preserved at relatively early stages in order to investigate the internal processes involved. Histories of several typical cases are given in the appendix.

Seventeen of the cases conform to the main type and do not differ materially from those considered in the last section.   As in the homopleural inverted limbs (p. 35), the degree and character of the reduplication vary much from case to case.   In some the digits alone are doubled, and at the other extreme we find two almost entirely separate limbs.   In thirteen individuals a second reduplicating limb formed on the anterior side of the original. These usually did not develop so completely as the limbs arising from the posterior buds, and the reduplication often involved only the distal part of the manus, with the digits more or less symmetrically placed.   The anterior reduplications are mirrored from the ulnar or ulnopalmar surface and occasionally from the

Figs. 67 to 70   Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 70.   Resulting limb reduplicated.   *N*, normal right limb bud; *TR*, transplanted limb bud; *PR*, primary member; *DU*, reduplicating member.   × 10.

Fig. 67   Dorsal view, five days after operation.   The transplanted bud already gives evidence of reduplication.

Fig. 68   Dorsal view, ten days after operation.   Reduplicating bud is large and in normal position.

Fig. 69   Dorsal view, sixteen days after operation.

Fig. 70   Dorsal view of specimen preserved thirty days after operation.

Fig. 71   Similar case (Exp. R. E. 71); ventral view, twenty-two days after operation.   × 10.

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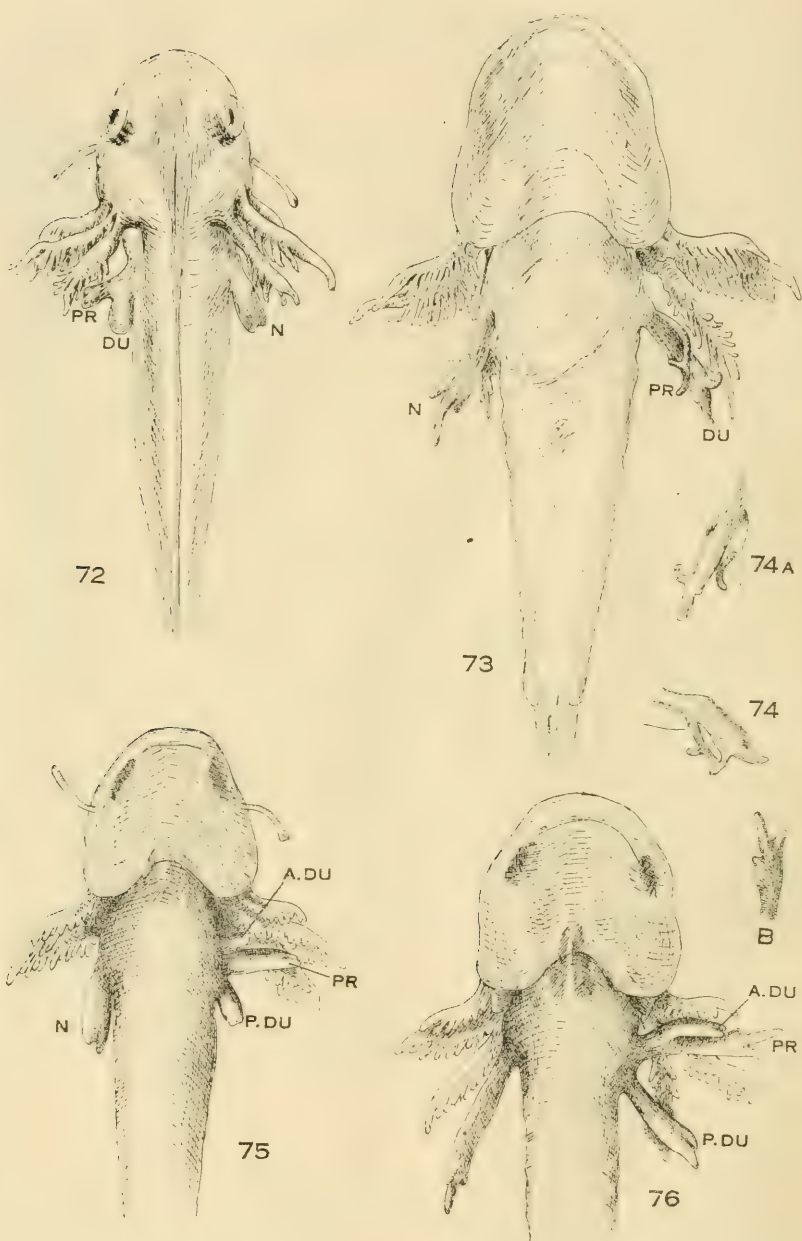
palm.   One case[52] had three almost complete separate appendages (figs. 75 and 76).

Two cases of the nineteen[53] gave rise to an anterior reduplicating bud only, which in both individuals was mirrored from the ulnopalmar surface.   Owing to the position of the reduplicating bud in front of the original heteropleural limb, it could not be brought into normal posture (fig. 77).

There remain to be considered the five cases in which the asymmetry of the transplanted bud was reversed.   These are of the utmost interest in showing how a secondary factor (reduplication) may so modify the result that the rules of symmetry seem not to hold.   They show more than any others the necessity of having complete histories in each case, for the manner in which the end result is reached is of cardinal importance for the correct interpretation of the process.   As stated above, these cases gradate into those in which duplicate limbs arise, so that the classification is somewhat arbitrary, the single-limb condition being a masked reduplication.   Like the others, they begin their development with growth of the bud in an anterior direction (figs. 83 and 86).   Then a posterior reduplicating bud makes its appearance, and the original bud is rapidly reduced (figs. 84 and 85 and 87 to 89) in relative importance, becoming a spur or nodule attached to the latter.   The history of a typical case is given on page 128.

Figs. 72 to 74   Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 74.   Reduplication with atrophic primary member.   *N*, normal right limb; *PR*, primary transplanted limb; *DU*, reduplicating member.   × 10.

Fig. 72   Dorsal view, twelve days after operation; the primary limb already appears as an appendage of the reduplicating member.

Fig. 73   Ventral view, twenty-one days after operation.

Fig. 74   Lateral view of transplanted limb.

Fig. 74A   Dorsal view of same.

Figs. 75 and 76   Orthotopic transplantation; right limb bud to left side (*het. dd.*).   Exp. R. E. 133.   Two almost perfect reduplicating members, one anterior (*A.DU*) and one posterior (*P.DU*) to the primary (*PR*).   The relations of these limbs are just as in the diagram, fig. 4B.   × 10.

Fig. 75   Ventral view, eleven days after operation.

Fig. 76   Ventral view, nineteen days after operation.   *B*, lateral view of posterior reduplicating member.

[52] R. E. 133, p. 127.   [53] R. E. 120 and 134.

In one individual[54] the growth in an anterior direction was well marked before the posterior reduplicating bud appeared (figs. 78 to 80). The anterior one was finally reduced to a spur, which, however, was considerably longer than in the next two cases of the series.[55] This is in reality the border-line case and might be
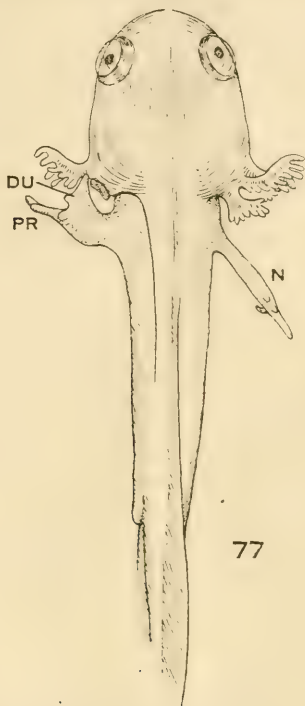
Fig. 77 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 134. The reduplicating member (*DU*) is anterior to the primary (*PR*). Preserved specimen, eighteen days after operation. × 10.

classed equally well as a reduplication. In one individual[56] the anterior bud was reduced to a slight scar, while in another[57] (figs. 90 to 92) it had only a very slight development and was soon entirely resorbed.

The cases in which the wounds were not cleaned (for the most part the earlier experiments made in 1911 and 1912) were seven-

[54] R. E. 108. [55] R. E. 77 and 69. [56] R. E. 91. [57] R. E. 95.
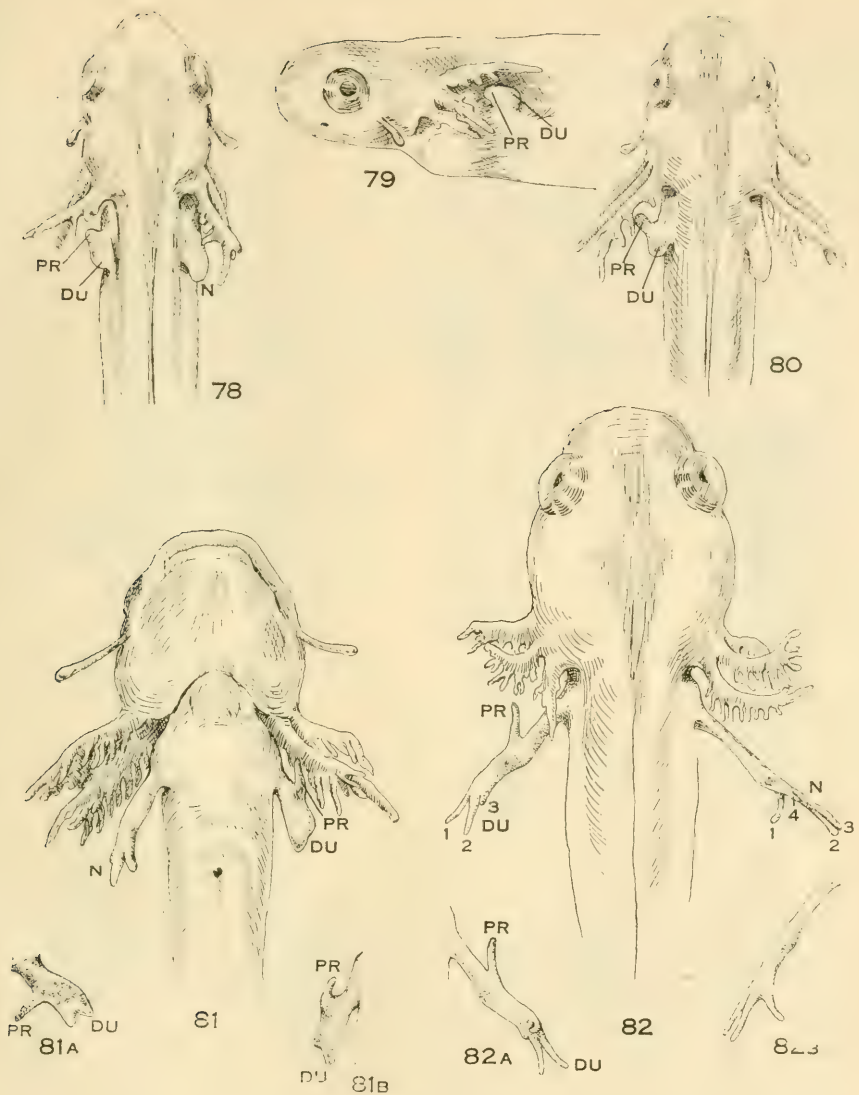
Figs. 78 to 82   Orthotopic transplantation; right limb bud to left side (*hct.dd.*).
Exp. R. E. 108.   Reduplicating member (*DU*) in normal position, developed at
expense of original (*PR*), which is reduced to a long spur.   *N*, normal right
limb; *1* to *4*, numbers of digits.   × 10.

Fig. 78   Dorsal view, six days after operation.   Reduplicating bud already
more massive, though less prominent, than the primary.

Fig. 79   Lateral view, six days after operation.

Fig. 80   Dorsal view, eight days after operation.

Fig. 81   Ventral view, fifteen days after operation.

Fig. 81A   Lateral view of limb.

Fig. 81B   Dorsal view of same.

Fig. 82   Dorsal view, thirty-three days after operation.

Fig. 82A   Ventral view of transplanted limb.

Fig. 82B   Ventral view of normal right limb.

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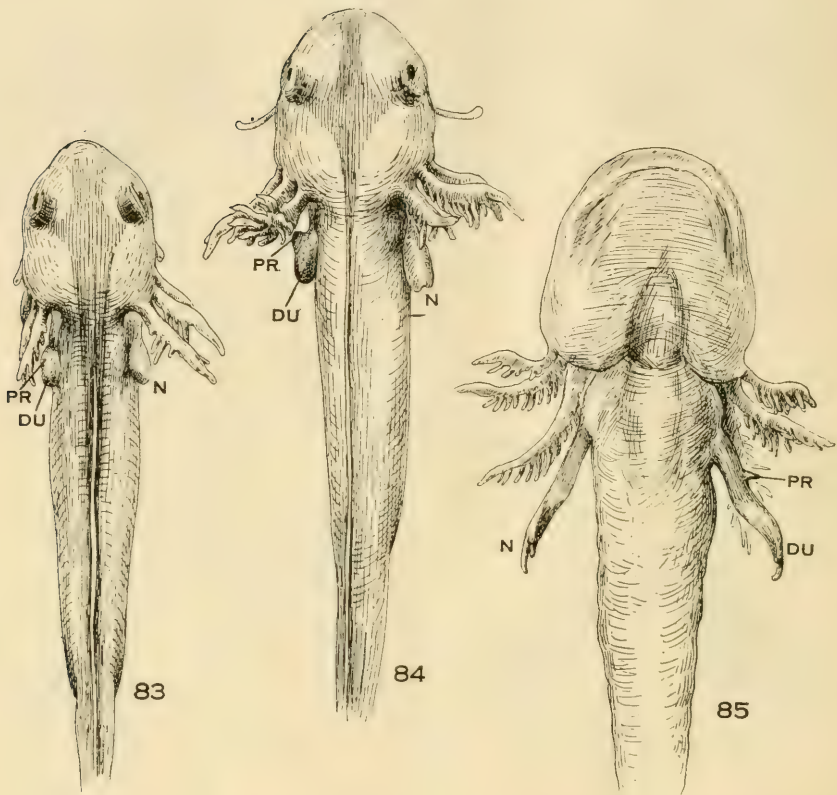
Figs. 83 to 85   Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 77.   Primary member (*PR*) reduced to a small spur on the reduplicating member (*DU*) which has become a normal left limb.   × 10.

Fig. 83   Dorsal view, eight days after operation.   Reduplication of transplanted bud already visible.   × 10.

Fig. 84   Dorsal view, eleven days after operation.   Reduplicating bud larger than primary.

Fig. 85   Ventral view, twenty-six days after operation.

Figs. 86 to 89   Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 69.   Primary member (*PR*) reduced to a nodule on the reduplicating one (*DU*).   × 10.

Fig. 86   Dorsal view, nine days after operation.

Fig. 87   Dorsal view, twelve days after operation.

Fig. 88   Dorsal view, sixteen days after operation.

Fig. 89   Ventral view, twenty-six days after operation.

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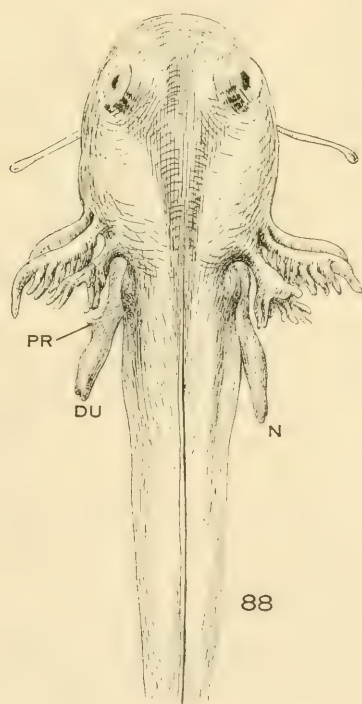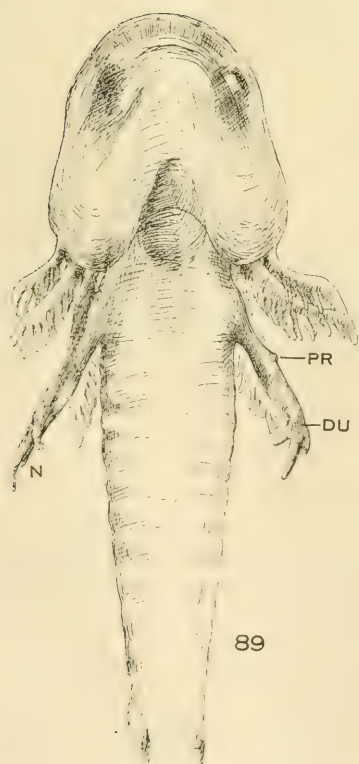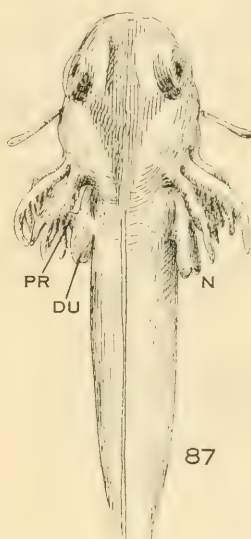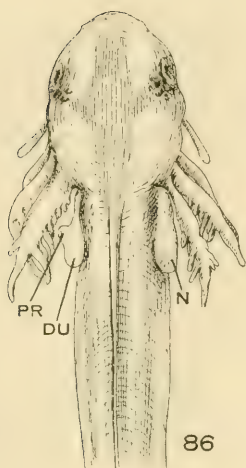
teen in number, in thirteen of which limbs developed. The distribution of these in the various groups does not show any significant differences from the cases with cleaned wounds. Eight (61.5 per cent) gave reduplications and three (23.1 per cent)
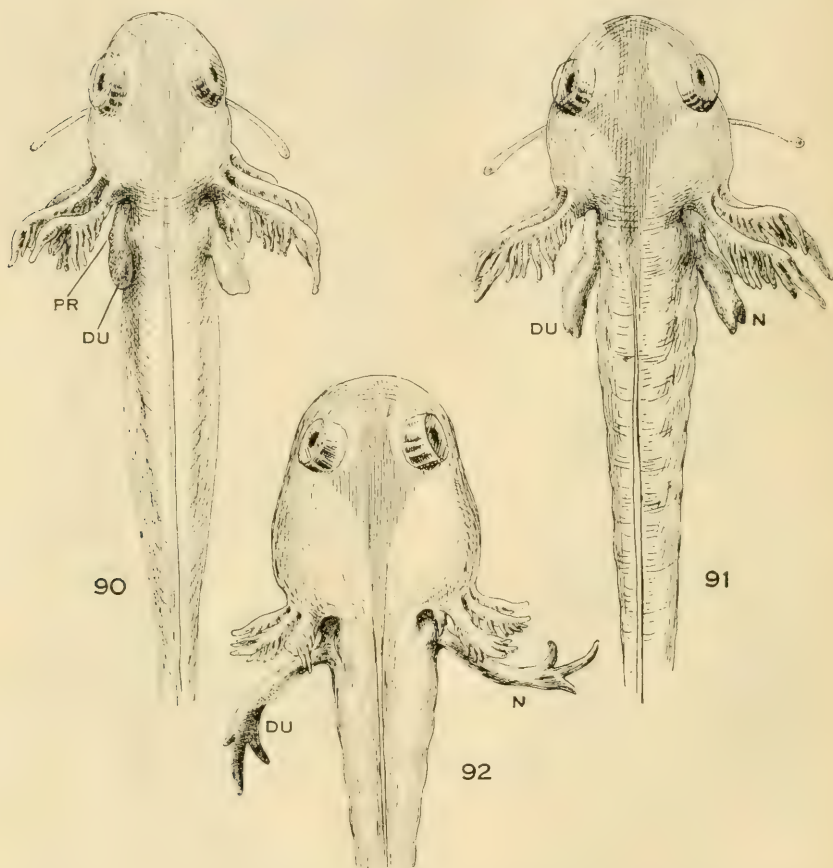
Figs. 90 to 92 Orthotopic transplantation; right limb to left side (*hct.dd.*). Exp. R. E. 95. Primary bud (*PR*) entirely obliterated; the reduplicating member (*DU*) a normal left limb. *N*, normal right limb. × 10.

Fig. 90 Dorsal view, ten days after operation; the primary bud (*PR*) shows as a slight nodule.

Fig. 91 Dorsal view, thirteen days after operation.

Fig. 92 Dorsal view, thirty-three days after operation. Owing to weakness of wrist and hand extensors, the larva has difficulty in bringing its hand to normal posture.

developed into reversed limbs. In at least two of the individuals reversal was brought about by reduplication. The third is uncertain. Of the two cases (15.4 per cent) recorded as developing without reversal, only one is clear. The other died at fifteen days and was lost, so that the notes made from the living specimen could not be verified.

10. Heteropleural transplantations, dorsoventral orientation. Twenty-six experiments were made in this group. In five out of the twenty-three individuals that lived the transplanted tissue was resorbed, and in two others the resulting appendage was imperfect or rudimentary, so that sixteen positive cases are available. Single limbs with reversed asymmetry developed in fifteen, and only one gave rise to a duplicate structure (table 2).

This group of cases shows that from the first the transplanted limb buds behave differently from those implanted in dorsodorsal orientation. When they begin to become prominent, they point dorsoposteriorly in most cases, though sometimes more sharply dorsally and frequently more laterally than the normal bud (fig. 93). As the bud grows, it thus occupies a nearly normal position, though it may continue for some time to project more sharply to the side or more dorsally than the normal limb (figs. 94 and 96). When the third and fourth digits develop, they are, however, not formed on the ventral border of the appendage, as they would be if the original asymmetry were preserved, but they come in on the dorsal border, just as in the normal hand of the side to which they were transplanted (figs. 2, 95, 98, 99, and 102). The palm of the hand, as in the normal individual, faces the body of the larva. Besides the one case in which reduplication actually occurred, there were three others in which slight indications of doubling appeared, only to disappear later, the more ventrally lying bud soon being resorbed. Histories of typical cases are given on page 128.

In all of these cases there was some retardation of development, and in some[53] it was very marked. A somewhat greater amount of tissue is lost by disintegration when the limb is placed dorsoventrally than when placed otherwise, since the bud does not

[53] *E. g.* R. E. 90.

fit into the wound so exactly. Besides this there seems to be a
time factor involved in the reversal, which would indicate that
the dorsoventral axis of the limb elements is slightly differenti-
ated, though not irreversibly so.
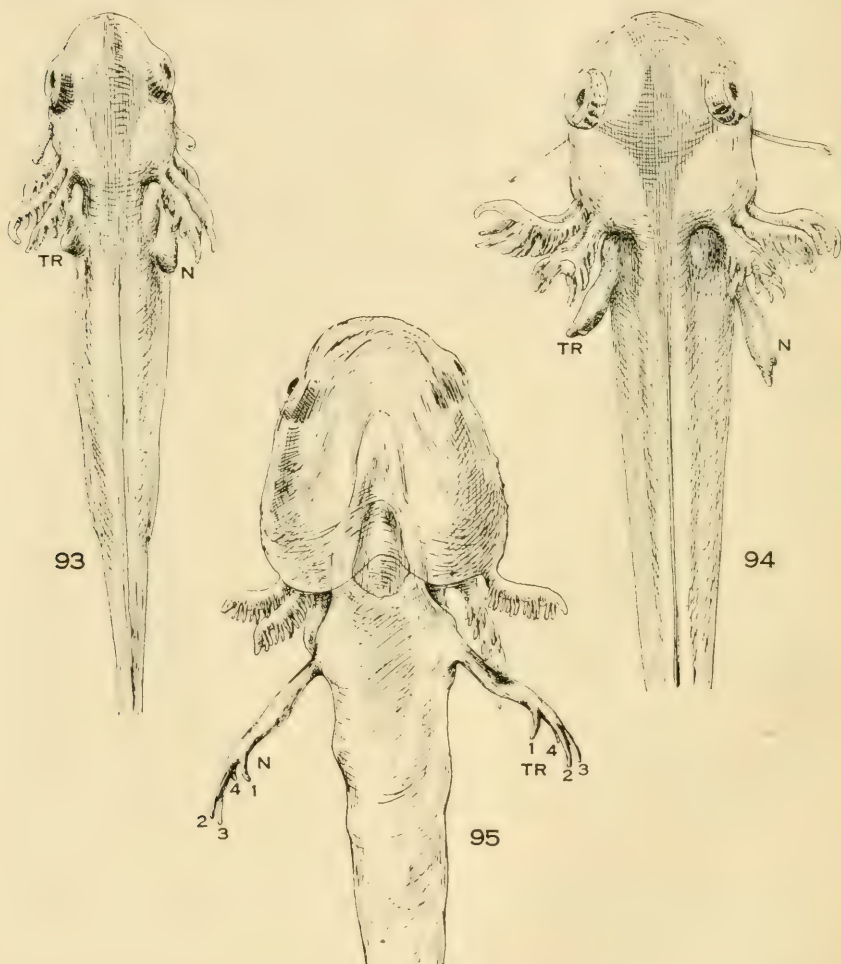
Figs. 93 to 95 Orthotopic transplantation; right limb to left side (*het.dv.*).
Exp. R. E. 80. Transplanted limb (*TR*) becomes a normal left. *N*, normal
right limb; *1* to *4*, numbers of digits.

Fig. 93 Dorsal view, six days after operation. Transplanted bud much
smaller than normal.

Fig. 94 Dorsal view, fourteen days after operation.

Fig. 95 Ventral view, forty-two days after operation.

The sole case in which a double appendage resulted[59] is interesting, inasmuch as it shows that the primary bud grows into a reversed limb, while the reduplicating bud has the original asymmetry (figs. 103 to 105). This is the opposite of the result obtained when the bud is implanted in dorsodorsal orientation. (History on p. 129.)

In the experiments with wounds not cleaned the proportion of reduplications is considerably larger—seven out of fifteen, or 46.7 per cent, as against one case in sixteen in the clean-wound group. There were eight cases (53.3 per cent) in which normal limbs with reversal of asymmetry developed, as against fifteen (94 per cent) in the case of the clean-wound experiments.

11. The shoulder-girdle in orthotopic transplantations. The above account has dealt only with the external features of the limb. The shoulder-girdle is likewise of interest.

As the heterotopic transplantations show, a small portion of the girdle surrounding the glenoid cavity always develops in connection with the grafted limb. After extirpation of the limb bud, however, the outlying regions of the girdle, including the suprascapula and portions of the procoracoid and coracoid, develop from cells that are left in the host.[60] It was to have been expected, therefore, that relations of harmony or disharmony would manifest themselves in the shoulder-girdle in orthotopic grafts. Study of serial sections of some of the cases shows that this is usually the case. Twelve individuals, belonging to three different groups have been examined in this way.

The three harmonic grafts (*het.dv*) all show girdles that are normal, except that they are somewhat underdeveloped. There has obviously been a union of host and graft tissues to form a normal whole, in spite of the fact that the transplanted bud was from the opposite side of the body.

The nine disharmonic grafts all show some form of irregularity, and in nearly all cases there is some sort of double girdle with reversal of the part that is derived from the graft. The condition of the girdle is complicated by the reduplication of the free

[59] R. E. 93.
[60] Cf. Detwiler, '18, p. 503, and Harrison, '18, p. 429.

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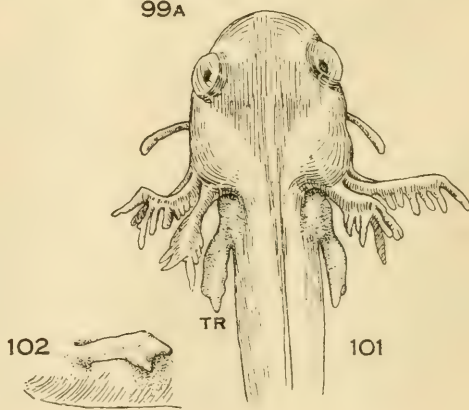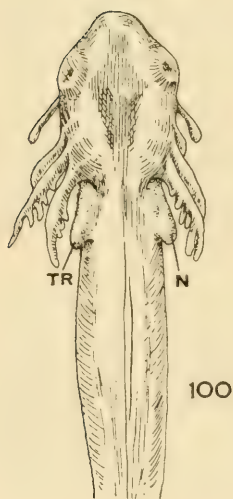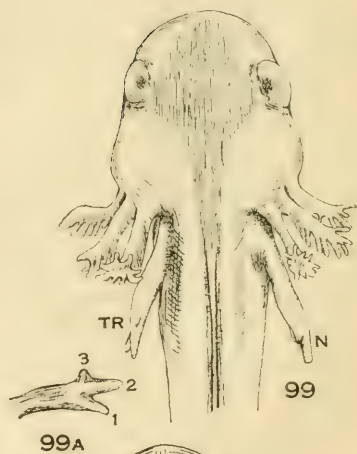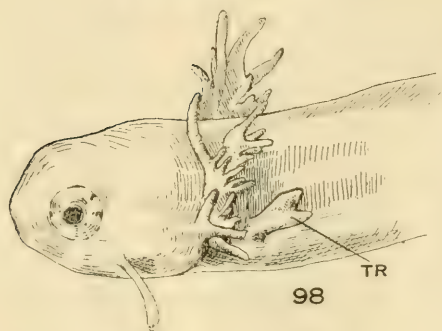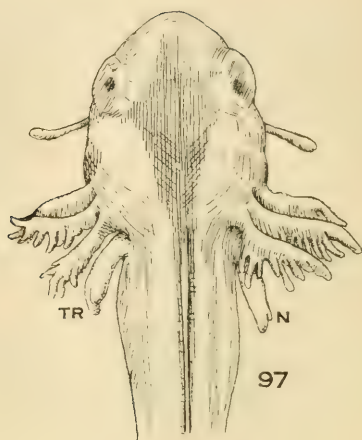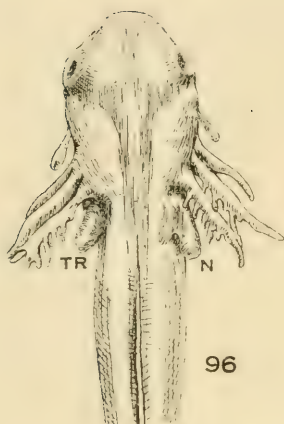
limb which takes place in most cases (table 2). It is more readily understood in the two cases in which a single limb of opposite asymmetry is present.

In the first of these,[61] in which a limb bud from the same side of the body was implanted in inverted position (p. 32, figs. 39 to 41), there are two entirely distinct shoulder-girdles. The anterior one has no connection with the other and is undoubtedly derived from the host, having the characteristics of girdles which develop after extirpation of the limb bud. The scapula and suprascapula are already joined in cartilaginous union with the procoracoid, but the coracoid is connected with the latter by ligament only. The girdle belonging to the transplanted limb is mainly posterior to the other, though there is some overlapping. It is large to have developed from a transplanted bud, but it has the characteristics of such. There is a distinct procoracoid process as well as a large coracoid, both of which project posteriorly from the glenoid cavity. This girdle is clearly reversed, as is the transplanted limb which is connected with it.

The other single disharmonic limb is the one developed from a bud taken from the opposite side of the body.[62] The limb itself is atrophic (fig. 64). The girdle is double, but the ventral parts of the two members are fused. The suprascapula, which is single and belongs to the host, is not connected with the rest. The

Figs. 96 to 99 Orthotopic transplantation; right limb to left side (*het.dv.*). Exp. R. E. 107.

Fig. 96 Dorsal view, eight days after operation. Transplanted bud (*TR*) smaller than normal (*N*) and more pointed.

Fig. 97 Dorsal view, thirteen days after operation.

Fig. 98 Lateral view, thirteen days after operation.

Fig. 99 Dorsal view, nineteen days after operation.

Fig. 99A Lateral view of transplanted limb. *1* to *3*, numbers of digits.

Figs. 100 to 102 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 116. Transplanted limb (*TR*) becomes a normal left. × 10.

Fig. 100 Dorsal view, seven days after operation. Transplanted bud only slightly smaller than normal.

Fig. 101 Dorsal view, thirteen days after operation.

Fig. 102 Lateral view, same age.

[61] I. E. 64.   [62] R. E. 87.
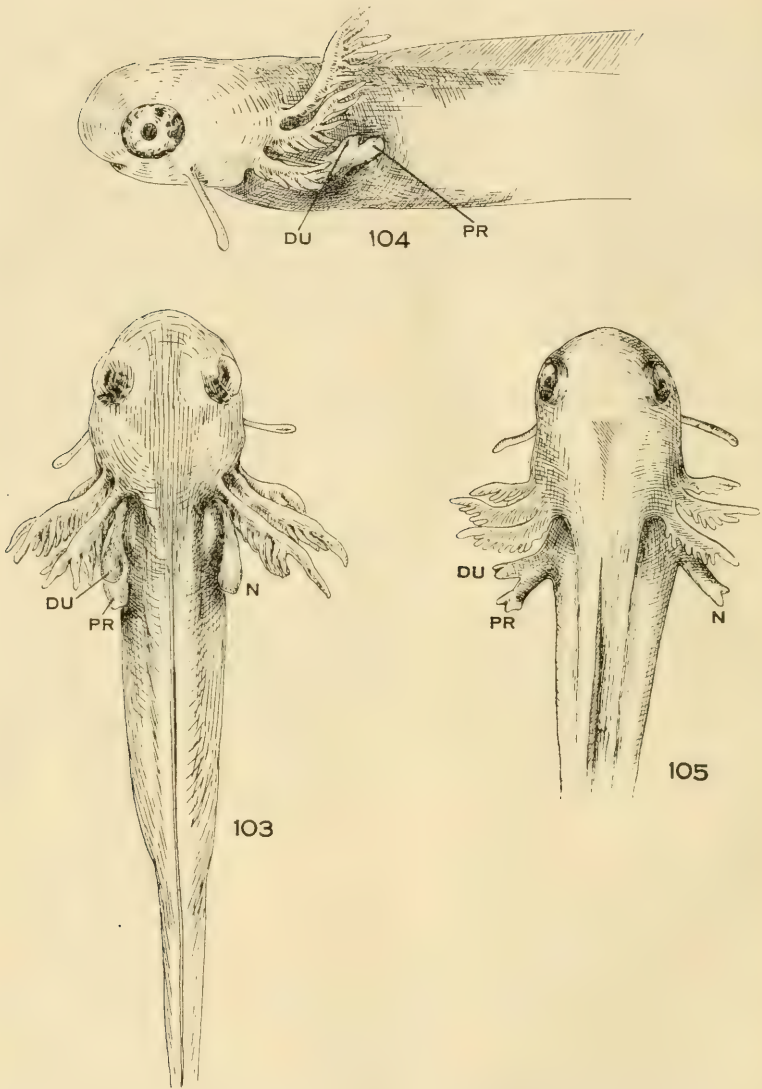
Figs. 103 to 105 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 93. Reduplication, the primary (*PR*) member being a left (reversed). *DU*, reduplicating member. × 10.

Fig. 103 Dorsal view, nine days after operation.

Fig. 104 Lateral view, same age.

Fig. 105 Dorsal view, preserved specimen, age fifteen days.

ventral part, which forms the glenoid cavity, is in fore and aft symmetry, with a coracoid and procoracoid process pointing in each direction. The posterior half of this cartilage has almost certainly developed in connection with the grafted limb and is reversed, while the anterior half is derived from the host.

In the disharmonic cases which have reduplicated limbs, the shoulder-girdles are on the whole less regular, owing to the complex articulations of the double appendages. One of them[63] (hom.dv) is, however, similar to the one first described in having two entirely separate girdles, one derived from the host and one from the graft. The suprascapula, procoracoid, and coracoid of the former are separate chondrifications, situated directly opposite the corresponding parts of the normal limb. The girdle of the transplanted limb has a broad flat glenoid cavity for articulation with the massive humerus. There is a large coracoid running ventrally from the joint, though without any very well-marked procoracoid. This girdle is placed some distance posterior to that of the host. Another of these cases[64] (hom.dv) is more like the second case described above, inasmuch as the dorsal element (suprascapula) is separate, while the two coracoids (from host and graft, respectively) are fused. The procoracoid of the host is a separate cartilage in this case. Two other cases[65] are of the same general type with fused coracoids, though they are rather too young to show all characteristics. Again, two others[66] have two scapulae with coracoids fused. There is only one case[67] that shows in sections practically no sign of doubling of the girdle, though even in this the coracoid region is thicker than normal and the glenoid cavity is large in correspondence with the more massive humerus.

To sum up: The shoulder-girdle in orthotopically grafted limbs is derived in part from the host and in part from the transplanted tissue. The former portion retains its normal asym-

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[63] I. E. 81.
[64] I. E. 93.
[65] I. E. 68 (hom. dv.) and R. E. 129 (het dd.).
[66] R. E. 77 (het. dv.) and R. E. 96 (het. dv.).
[67] I. E. 60 (hom. dv.).

metry, while the latter behaves in accordance with the rules governing the asymmetry of transplanted limbs. In the disharmonic combinations the portions of the girdles derived, respectively, from the two sources may fuse together or may remain entirely separate. In the harmonic combinations they unite to form a single normal girdle.

12. Summary of the results of orthotopic transplantations. The orthotopic transplantations develop according to the same rules as the heterotopic. In the homopleural dorsodorsal and the heteropleural dorsoventral groups rules 1 and 2 (p. 4) are very closely followed. In the former the limb buds, being right side up, retain their normal asymmetry; and in the latter, being upside down, they reverse it. In both groups this results in limbs which correspond to the side on which they are implanted (harmonic combinations).

In the other two groups the primary single limbs which develop do not correspond to the organic environment, since the homopleural graft, when placed upside down, becomes reversed, and the heteropleural graft right side up retains its original prospective asymmetry. In these combinations, which have been called disharmonic, single limbs are, however, the exception. It is here that rule 3 comes into play. Reduplications occurred in 71.1 per cent of the cases in the homopleural dorsoventral group and in 80.6 in the heteropleural dorsodorsal. The former includes only one case of single limb reversed. In this class are also five cases of reversed single limbs, which are fundamentally the same as reduplications, the original limb having been suppressed or resorbed. The disharmonic relation thus augments immensely the tendency to reduplicate. In the case of the heterotopic grafts, on the contrary, the greater proportion of reduplications occurs in the harmonic combinations. This curious fact will be discussed below (p. 107). The ten cases of non-reversed single limbs which resulted from homopleural inverted buds are, as already pointed out, exceptional in that the limb regained its normal posture gradually during development by rotation at the base.

C. Superposed limb buds

In the preceding study of transplanted limbs certain experiments were described, which showed that the mesoderm from two limb buds, when fused together, would develop into a single normal limb. At first larger than normal, the size of such a limb is soon regulated. In the former communication only those experiments were considered in which the orientation of the superposed bud was normal (*hom.dd*). The effect of the orientation of the graft will now be taken up.

TABLE 3

Superposed limbs. Summary of results

OPERATION	NUMBER OF EXPERIMENTS		NORMAL SINGLE LIMBS		NORMAL SINGLE WITH REDUCED REDUPLICATION		REDUPLICATED	
	Total	Positive	Number	Per cent	Number	Per cent	Number	Per cent
Hom. dd	5	5	5	100	0	00	0	00
Hom. dv	5	5	1	20	0	00	4	80
Het. dd	6	5	0	00	1	20	4	80
Het. dv	9	5	5	100	0	00	0	00
Total	25	20	11	55	1	5	8	40

The experiments are summarized in table 3. There were twenty-five operations, of which twenty are available for the analysis. Two of the combinations, the ones which the ordinary transplantations have shown to be harmonic (homopleural dorsodorsal and heteropleural dorsoventral) yielded only normal appendages (ten cases). The two disharmonic combinations (homopleural dorsoventral and heteropleural dorsodorsal) yielded reduplications in nine cases out of ten. One case, in which one member of the duplicate limb was reduced to a spur, is included among the reduplications.

13. Homopleural transplantations, dorsodorsal orientation. In this group development went forward with a minimum of disturbance. The only abnormal feature to note is the large size of the double bud in certain individuals. In several of the cases
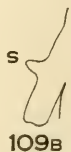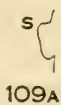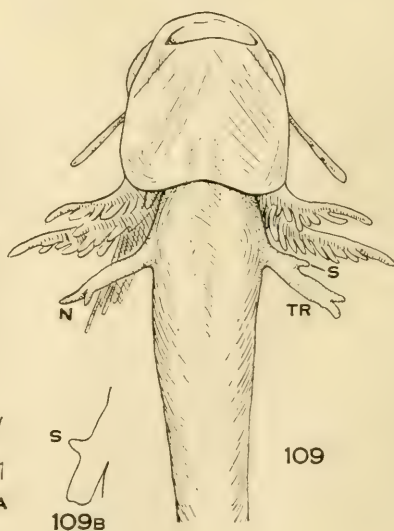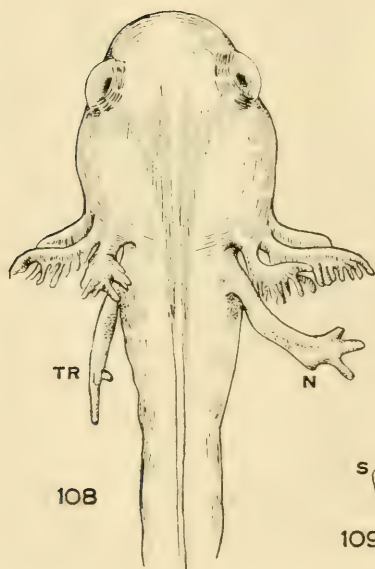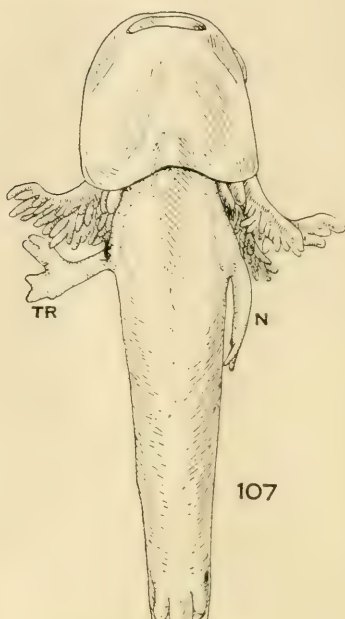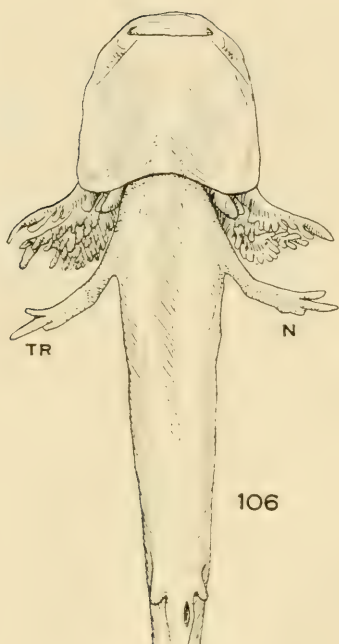
the difference from normal size persisted for twelve or more days,[63] gradually diminishing during that period (fig. 106). In others the difference was less marked, though in all some difference in favor of the limb on the operated side was noted.

14. Homopleural transplantations, dorsoventral orientation. Four out of the five cases in this group gave rise to reduplications in the grafted limb.

The reduplications vary. One[69] is a typical case of radial mirroring of the lower part of the forearm and hand (fig. 107). Another[70] is similar, except that the anterior member is itself reduplicated, the hand being a nearly symmetrical complex with four digits. These two individuals are in every respect like those cases of reduplication resulting from simple inverted limb buds, in which the primary member is reversed and is accompanied by a non-reversed twin which takes up the normal position. In two other cases[71] the reduplication is less and is of a character not necessarily attributable to disharmonic combination, though there is nothing to indicate that it is not due to such a cause. Principally the digits are involved (fig. 108).

In the remaining case[72] a normal limb developed. This one and possibly also the two foregoing are analogous to those cases of simple transplantation in which the inverted limb bud develops into a normal limb without reversal by means of rotation (p. 40).

Fig. 106 Superposed limb bud; right limb bud to right side, normal position (*hom.dd.*). Exp. S. E. 3. Normal limb (*TR*) on operated side. *N*, normal left limb. Preserved specimen, ventral view, eighteen days after operation. $\times$ 10.

Fig. 107 Superposed limb bud; right limb to right side inverted (*hom.dv.*). Exp. S. E. 18. Reduplicated appendage (*TR*) on operated side. Preserved specimen, ventral view, eighteen days after operation. $\times$ 10.

Fig. 108 Superposed limb bud; right limb bud to right side inverted (*hom.dv.*) Exp. S. E. 9. Operated limb (*TR*) normal except for reduplication of second digit. $\times$ 10.

Fig. 109 Superposed limb bud; right limb bud to left side (*het.dd.*). Exp. S. E. 6. Reduplication with heteropleural member reduced to spur (*S*). $\times$ 10.

Fig. 109A Outline of limb bud from above. Five days after operation (freehand sketch).

Fig. 109B Same, eleven days after (free-hand sketch).

[63] S. E. 3.   [69] S. E. 18.   [70] S. E. 2.   [71] S. E. 9 and 14.   [72] S. E. 10.
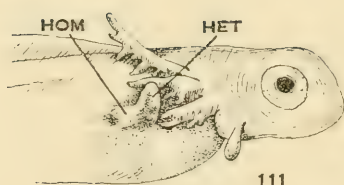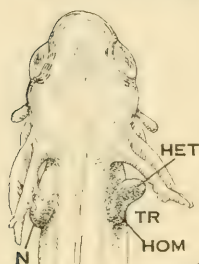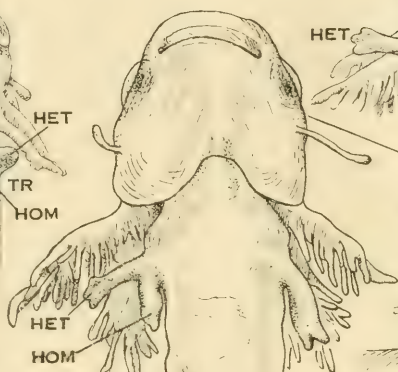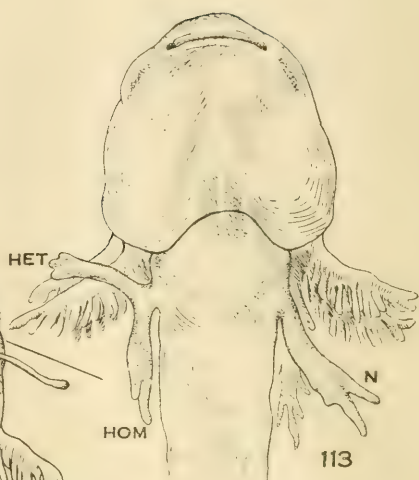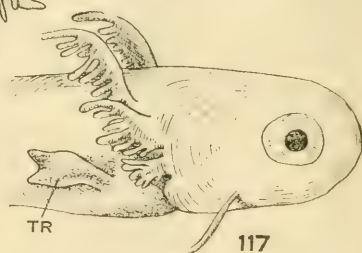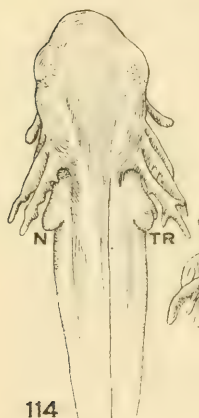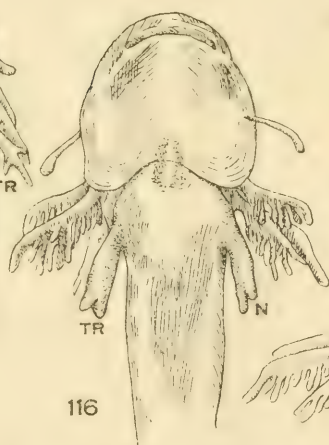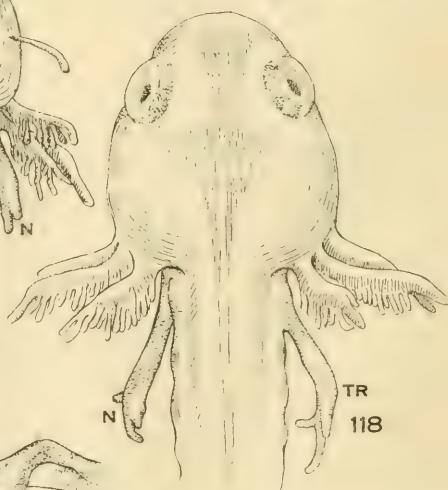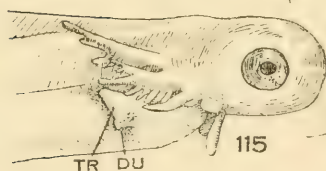
15. Heteropleural transplantations, dorsodorsal orientation. In this group the results of five experiments all fall clearly within the same category. A normal appendage in approximately normal position developed, and this had a reduplicating member attached to its radial border. The differences between the cases consist in the extent of reduplication and the degree of development attained by the disharmonic (anterior) member. The most extreme cases are one[73] in which the heteropleural member is reduced to a spur (fig. 109), and one[74] in which there is almost complete doubling from just below the shoulder down, the heteropleural member being, however, atrophic (figs. 110 to 113). Two of the three remaining cases[75] are very similar to one another, the anterior member with two digits arising from near the elbow. In the third individual the hand is reduplicated[76] externally, and the whole arm is somewhat shorter and thicker, indicating some degree of internal reduplication.

16. Heteropleural transplantations, dorsoventral orientation. The five cases in this class all gave normal limbs (figs. 117 and 118). Three of them showed slight indication of doubling (figs. 114 and 115) in the early stages of development (four or five days after operation), but the more ventral-lying prominence had disappeared at the time of the next observation in each individual

Figs. 110 to 113 Superposed limb bud; left to right side (*het.dd.*). Exp. S. E. 12. Reduplication. *N*, normal left limb; *TR*, operated limb; *HET*, heteropleural member; *HOM*, homopleural member.

Fig. 110 Dorsal view, five days after operation.

Fig. 111 Lateral view, same age.

Fig. 112 Ventral view, ten days after operation.

Fig. 113 Ventral view, seventeen days after operation.

Figs. 114 to 119 Superposed limb bud; left to right side (*het.dv.*). Exp. S. E. 11. Operated limb (*TR*) of large size; *N*, normal unoperated limb. × 10.

Fig. 114 Dorsal view, five days after operation.

Fig. 115 Lateral view, same age. A distinct nodule or bud (*DU*) on ventral border of limb was soon afterward resorbed.

Fig. 116 Ventral view, ten days after operation.

Fig. 117 Lateral view, same age.

Fig. 118 Dorsal view, seventeen days after operation.

Fig. 119 Lateral view of limb, same age.

[73] S. E. 6.   [74] S. E. 12.   [75] S. E. 8 and 21.   [76] S. E. 15.

(figs. 116 and 117). A sixth case[77] is inconclusive owing to general weakness of embryo, but it is not inconsistent with the results in the other cases. A typical history is given in the appendix (p. 131).

Though more than the normal amount of material is present in the composite limb bud, in two of the cases of this group the developing limb is recorded at first as slightly smaller than the normal. In one case no difference in size was noted, while in two the limb on the operated side is noted as larger. In the other harmonic combination (homopleural dorsodorsal) all cases showed the limb on the operated side to be somewhat larger in size.

17. Discussion of experiments with superposed limb buds. The principal differences between these experiments and those of simple transplantation are that in the former the tissue available for the formation of the limb is approximately double in amount, and there is a mixture of tissues having two different orientations except in the one group, homopleural dorsodorsal. In the harmonic combinations the amount of tissue is so regulated that after a time size-differences disappear. The amount of tissue is, moreover, never quite double that of the normal limb because of the material lost by the operation and of the general retardation of growth due to the same cause. In the case of the heteropleural dorsoventral grafts, which are classed as harmonic, some readjustment must be necessary, as shown by the amount of retardation.

In all of the disharmonic combinations there is a mixture of tissues differently oriented and with different prospective meaning as regards the particular asymmetry of the future limb. The twin limbs that arise are, therefore, not necessarily due to reduplication by budding, as they must be in the simple transplantations, but probably in part at least to the circumstance that one of the pair develops out of the original limb bud, while the other is from the transplanted tissue.

[77] S. E. 16.

D. *Transplantation of half buds*

Partly as a further test of the question of equipotentiality and partly to study more thoroughly the effect of harmonic and disharmonic combinations, a series of experiments with half limb buds was instituted. Instead of removing the whole circular disc comprising the limb rudiment, a semicircular piece was cut out, the wound bed carefully cleaned, and the removed portion replaced by a piece of similar size and shape from another limb bud. Considering only vertical and horizontal halves and replacing vertical only with vertical and horizontal only with horizontal, there are sixteen different experiments possible, which have been numbered in the diagram (fig. 120) from 1 to 16.

There are five different pairs of attributes, which appear as alternatives in the operations. Thus the transplanted half bud is either—1) homopleural (*hom.*) or heteropleural (*het.*); 2) upright (*dd.*) or inverted (*dv.*); 3) homogeneous (*homogen.*) or heterogeneous (*heterogen.*); 4) vertical (*vert.*) or horizontal (*horiz.*); 5) anterior (*ant.*), dorsal (*dors.*) or posterior (*post.*), ventral (*vent.*).

This aggregation would consist of 2^5 or thirty-two classes, were it possible to combine the attributes of operation independently without restriction, as would be the case were the pieces rectangular. Since, however, they are semicircular they fit in only half the cases, and the total is therefore reduced to sixteen. All of the possible experiments have been performed.

If both halves of the disc are considered movable, further possibilities open up. There would then be thirty-two different combinations, which, however, in eight cases would be practically identical with the experiments where the whole disc is transplanted. None of these experiments have been performed, since the technical difficulties would be at least doubled, and, as far as the study of either of the questions at issue is concerned, they would offer no advantage over those in which only one half of the bud is transplanted. Again, were the limb disc homogeneous, either or both halves could be turned inside out and then one hundred and twenty-eight different combinations would be possible. These are precluded, as in the case of the whole discs, by the

impossibility of grafting successfully pieces with the mesoderm turned toward the outside and by the difficulty of handling pieces of mesoderm free from ectoderm without disturbing their arrangement. Perhaps we may consider ourselves fortunate in being subject to such restrictions.
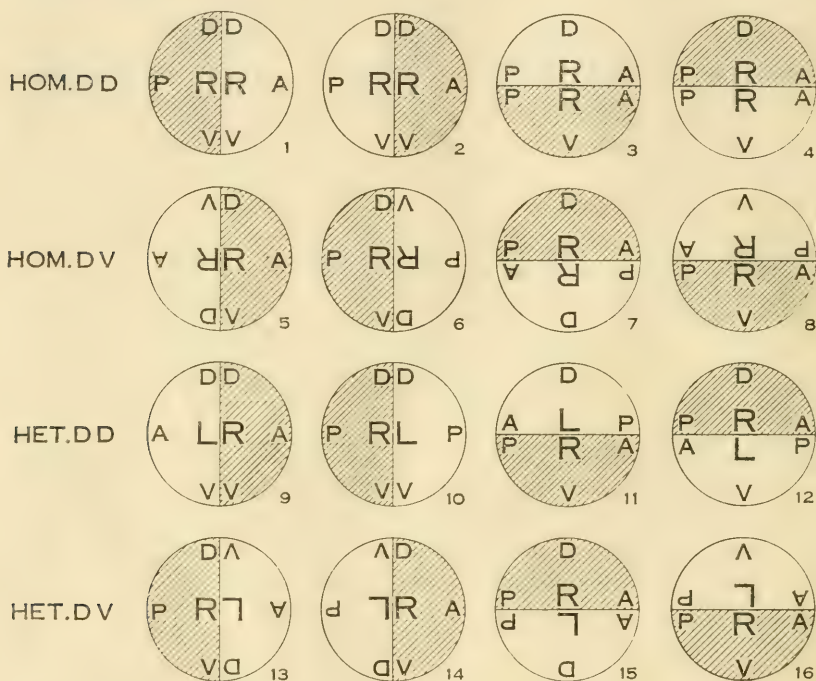
Fig. 120 Diagram showing the sixteen possible combinations (1 to 16) obtainable by transplanting half limb buds. The shaded area signifies the stationary half, the clear area the transplanted half. *R*, right; *L*, left; *D*, Dorsal; *V*, ventral; *A*, anterior; *P*, posterior. The operations are represented as on the right side of the embryo.

Returning to the experiments actually carried out (fig. 120), we find that four of them consist merely in replacing the excised piece with another of exactly the same kind in normal orientation. These serve, therefore, as controls for testing the effect of the operation as such on the further course of development. It is also seen that half of the combinations are harmonic and half disharmonic (p. 8). Half are of course homogeneous or com-

posed of two similar halves, while the other half are heterogeneous. Of the former, six belong to the disharmonic group and only two to the harmonic, while of the latter the reverse is the case, a circumstance that affects the proportionate results of the experiments.

The effect of removal of the various halves of the limb rudiment has already been described (Harrison, '18). As shown by such experiments, any half of the limb bud can give rise to a whole limb, though quantitatively the material is eccentrically distributed, there being more limb-forming tissue in the dorsal and anterior halves than in the ventral and posterior halves, respectively. Accordingly, four of the homogeneous combinations would have somewhat less than the normal amount of tissue, while four would have a little more. In the later experiments an attempt was made to compensate for this by not cutting the area exactly in half.

Owing to the large number of combinations in the experiments, it has not been possible to perform a sufficient number of each, for accurate statistical treatment. The number is sufficient, however, to compare the more comprehensive groups; for instance the homogeneous with the heterogeneous and the harmonic with the disharmonic.

Seventy-nine operations were done, sixty-eight healing successfully. Badly defective limbs developed in but four cases, so that sixty-four remain for the purpose of the analysis. These experiments are summarized in table 4.

From the results of transplanted whole limbs we should expect the following to take place; the harmonic combinations should give rise to simple normal limbs, the disharmonic to reduplications. The homogeneity or heterogeneity of the combination should not be expected to make any difference in view of the other tests of the equipotentiality of the system. These expectations were in the main realized, probably in fifty-five out of the sixty-four cases (85.9 per cent) (table 7). There are, however, sources of confusion, which in certain cases make several interpretations possible, and which for this and other reasons must not be overlooked. For example, it is known from experiments with whole

limb buds, that a normal limb may arise from a disharmonic combination by the suppression of the original bud or by its reduction to a mere excrescence on the reduplicating member, which latter may develop into a normal limb. Eight of the ten normal cases which would otherwise appear anomalous may certainly be thus explained, and possibly the remaining two. It has been found

TABLE 4

Transplantation of half limb buds. Summary of results of actual experiments

			OPERATION					RESULTING LIMB			
Number	Side of origin of graft	Orientation	Composition	Direction of halving	Designation of transplanted half	Normal	Normal by resorption	Reduplicated	Abortive	Dead
1	hom.	dd	heterogen.	vertical	ant.	2	0	0	0	1
2	hom.	dd	heterogen.	vertical	post.	2	0	0	0	1
3	hom.	dd	heterogen.	horiz.	dors.	2	0	0	0	0
4	hom.	dd	heterogen.	horiz.	vent.	2	0	0	0	0
5	hom.	dv	homogen..	vertical	ant.	0	2	2	0	1
6	hom.	dv	homogen.	vertical	post.	0	0	5^2	0	2
7	hom.	dv	homogen.	horiz.	dors.	0	2	2	0	0
8	hom.	dv	homogen.	horiz.	vent.	0	0	4	1	0
9	het.	dd	homogen.	vertical	ant.	2	2	0	3	1
10	het.	dd	homogen.	vertical	post.	0	1	3^1	0	0
11	het.	dd	heterogen.	horiz.	dors.	0	0	5^1	0	1
12	het.	dd	heterogen.	horiz.	vent.	0	0	4	0	1
13	het.	dv	heterogen.	vertical	ant.	4	0	1	0	1
14	het.	dv	heterogen.	vertical	post.	4	0	0	0	2
15	het.	dv	homogen.	horiz.	dors.	5	0	2	0	0
16	het.	dv	homogen.	horiz.	vent.	6	0	0	0	1
Total number of cases, 79; positive cases, 64....						29	7	28	4	11

[1] Includes one case of anomalous reduplication.

[2] Includes two cases of anomalous reduplication.

also that almost any transplantation or even simple defect experiment may sometimes bring about reduplication. The three anomalous reduplications, being slight, are probably of this class. A further source of error might arise from the circumstance, that either the grafted or the stationary half may in certain cases be solely responsible for the limb that develops; for it is known, on the one hand, that any graft may be resorbed and, on the other,

that when half the disc is excised, complete suppression of development may sometimes result, probably through accidental injury to the remaining part. The result of the former contingency would be confusing, owing to the development of a normal limb in place of a reduplication.

In connection with these questions it must also be borne in mind that the cases of union of two disharmonic halves differ, with respect to the disharmony of the combination, from those in which the limb-bud has been transplanted as a whole. In the former only one half of the rudiment is involved, the other being in all cases harmonic with the surrounding tissues. We are, therefore, dealing with a rudiment that is disharmonic in itself, while in the case of the whole limb the transplanted bud is harmonic in itself, though disharmonic with respect to the organism as a whole. This might possibly give rise to some differences in the results in the two classes of experiments.

In view of these considerations, we should not expect the transplantation of half buds to give such clear-cut results as the experiments with whole ones. On the other hand, it must not be overlooked that the sources of confusion above enumerated, while accounting for nearly all of the anomalies, also render less cogent the cases which conform to the rules. Nevertheless, after taking all circumstances into consideration, it can scarcely be doubted, that the experiments with half discs do afford a valuable confirmation of the results obtained from the other experiments.

18. Homopleural transplantations, dorsodorsal orientation. The eight cases of homopleural grafts in upright orientation (*hom.dd*), two involving each half of the bud, all resulted in normal limbs, as was to have been expected, for this operation is nothing more than replacing an excised portion with one exactly similar. Only slight retardation of development is recorded in some of the cases.

19. Homopleural transplantations, dorsoventral orientation. The nineteen experiments with homopleural grafts in inverted position (*hom.dv*) resulted, in accordance with expectation, in a large number (fifteen) of duplicities[78] (figs. 121 and 122). The

[78] H. E. 29 and 31.

remaining four cases were normal.   In the early stages, however,
all of the latter gave evidence of reduplication (fig. 123).   The
limb bud, when it first appeared, showed two distinct nodules or
prominences, one of which developed into a normal limb.   In
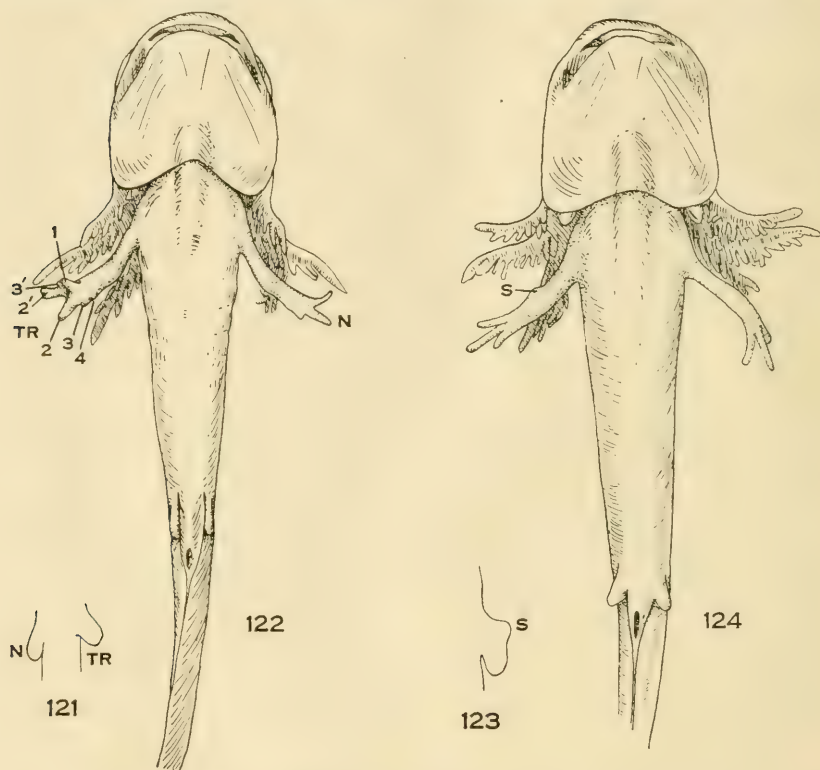
Figs. 121 and 122 Transplantation of half limb bud (comb. 6, fig. 120); pos-
terior right to anterior right (*hom.dv.*).   Exp. H. E. 31.   Partial reduplication
of hand, mirror plane being radiodorsal.   Arm and medial hand homopleural.
1 to *4*, numbers of digits of main hand; *2'*, *3'*, digits of reduplicating member.

Fig. 121   Outline of normal (*N*) and operated (*TR*) buds from above, nine
days after operation (free-hand sketch).

Fig. 122   Ventral view, preserved specimen twenty-one days old.   × 10.

Figs. 123 and 124   Transplantation of half limb bud (comb. 7, fig. 120); dorsal
right to ventral right (*hom.dv.*).   Exp. H. E. 2.   Operated limb slightly thicker,
reduplicating member reduced to a nodule (*S*).

Fig. 123   Outline of operated limb, dorsal view eight days after operation
(free-hand sketch).

Fig. 124   Ventral view preserved specimen, twenty days old.   × 10.

three cases the other prominence persisted also, at the elbow in one[79] in the form of a spur (fig. 124), and as a nodule at the shoulder in two others.[80] In the remaining case[81] all external traces of reduplication disappeared.

On the other hand, two of the cases of reduplication are of an anomalous nature and cannot be regarded as conforming to the rule. Both of these were experiments in which the anterior half of the limb bud was replaced by a posterior half. We should expect in such a case to find posteriorly a homopleural member developed out of the stationary portion of the bud, while anteriorly there should be a reversed limb which might itself be reduplicated. The opposite is, however, true. In both cases the anterior member is not reversed. The posterior member is reversed (a left) in one case[82] (fig. 126) and in the other[83] it is itself double, the anterior portion being reversed and the posterior homopleural (fig. 125).

The operated limb in all of these cases was composed of two homogeneous halves.

Histories of typical cases are given in the appendix (p. 132).

20. Heteropleural transplantations, dorsodorsal orientation. This combination, being disharmonic, yielded out of seventeen cases twelve duplicities (figs. 127 and 129) and three limbs that became normal by reduction of the reduplicating member (fig. 130). In two individuals[84] normal limbs resulted without external evidence of incipient doubling, and two of the reduplications, in one of which both members are of the same side in linear series, are of an anomalous nature. This makes four cases out of seventeen that do not follow the rule. Two of the combinations are heterogeneous; all of these conform to the rule except one of the anomalous reduplications. The other three non-conforming cases belong in the homogeneous class, and it is interesting that all of the normal cases in this group resulted from the combination of two like halves.

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[79] H. E. 2.
[80] H. E. 18 and 21.
[81] H. E. 4.
[82] H. E. 13.
[83] H. E. 5.
[84] H. R. E. 43 and 44.

125
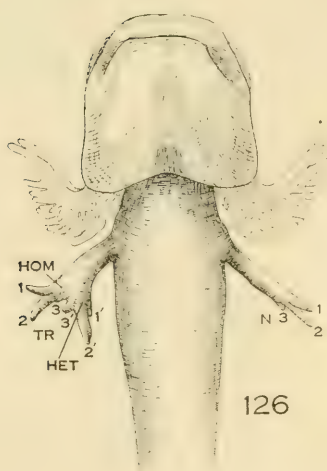
126
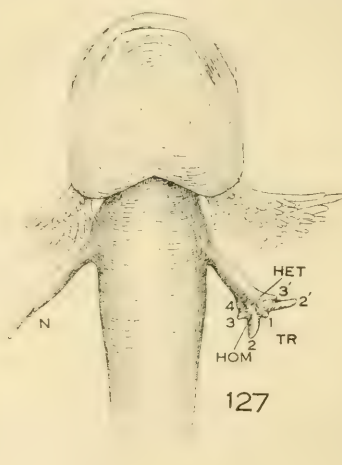
127

Fig. 125 Transplantation of half limb bud (comb. 6, fig. 120); posterior half right to anterior right (*hom.dv.*). Exp. H. E. 5. Two limbs, the posterior of which has a double hand. Anterior member an almost normal right; of the two parts of the posterior member, the anterior one is a left hand and the posterior, a right. × 10.

Fig. 126 Same operation (comb. 6, fig. 120) (*hom.dv.*). Exp. H. E. 13. Ulnar reduplication! *HOM*, homopleural hand; *1* to *3*, digits of same; *HET*, heteropleural hand; *1'* to *3'*, digits of same; *N*, normal (unoperated) left limb × 10.

Fig. 127 Transplantation of half limb bud (comb. 10, fig. 120); posterior half right to anterior left (*het.dd.*). Exp. H. R. E. 1. Double hand. *HOM*, homopleural hand with digits (*1* to *4*); *HET*, heteropleural hand with digits (*2'* to *3'*). × 10.

The double limbs are of various degree and kind. The least involved is one in which only the first digit is doubled.[85] In this individual the ventral half of the limb was replaced by a ventral half of the opposite side, and in all probability very little limb material was actually transplanted, since, in the embryo from which the graft was taken, the operated limb developed almost as rapidly as the normal. In five other cases[86] (fig. 127) the whole hand is involved, with indications that in three of these at least[87] the internal reduplication extends farther proximally. In two cases the fore arm and hand[88] (figs. 128 and 129) are externally double, and in one,[89] which was not fully developed when preserved, doubling would probably have shown from near the shoulder down. In two of the individuals[90] there are secondary reduplications. In the two anomalous cases[91] (figs. 131 and 132) there are two entirely separate limbs.

Histories are given on page 134. Unfortunately, external observation does not always reveal the relations of each of the two halves of the bud to the developing members.

21. Heteropleural transplantations, dorsoventral orientation. Out of twenty-two successful experiments in this group nineteen resulted in the development of normal limbs (fig. 133), which is according to rule, and only three gave rise to reduplications (fig. 134). Two of the latter[92] involved only the radial digits, in which palmar reduplication was present, the limbs being otherwise normal. In the remaining one a bifurcated appendage arose, but the dorsal branch remained merely as a spur attached to the main limb, which was normal though undersized and with slight syndactyly.

In one case,[93] which has been classed as normal, a filamentous appendage probably not a limb, developed a short distance ventral to the main limb, which was normal though slightly shorter.

[85] H. R. E. 30.

[86] H. R. E. 1, 15, 46, 47, and 48.

[87] H. R. E. 15, 46, and 47.

[88] H. R. E. 5 and 21.

[89] H. R. E. 28

[90] H. R. E. 21 and 47.

[91] H. R. E. 9 and 20.

[92] H. R. E. 10 and 16.

[93] H. R. E. 12.

This group of experiments is interesting because two of the combinations (ventral half in place of dorsal and dorsal in place of ventral) are homogeneous. Out of thirteen such cases, normal limbs developed in eleven.

For histories of representative cases see appendix (p. 136).
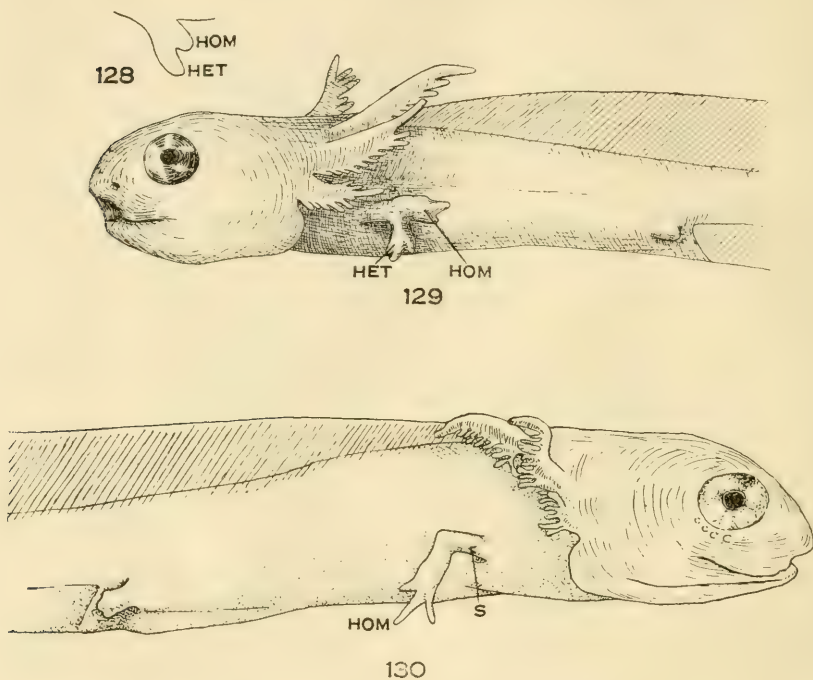
Figs. 128 and 129 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half right to dorsal left (*het.dd.*). Exp. H. R. E. 5. *HOM*, homopleural member; *HET*, heteropleural member.

Fig. 128 Outline of limb from above, ten days after operation (free-hand sketch).

Fig. 129 Preserved specimen lateral view, nineteen days old. × 10.

Fig. 130 Transplantation of half limb bud (comb. 9, fig. 120); anterior half left limb bud to posterior right (*het.dd.*). Exp. H. R. E. 11. Normal limb with small spur (*S*). × 10.

22. Discussion of experiments with half buds. In order to establish the conclusion stated in the introduction to this section, that it is the harmony or disharmony of the half-and-half combi-

nation and not one of the particular qualities of the operation that determines whether normal or reduplicated limbs arise, it will be necessary to examine the numerical results of the experiments more carefully.

If we take the actual figures of the experiments and examine the qualities in pairs, we find the actual number of each class and
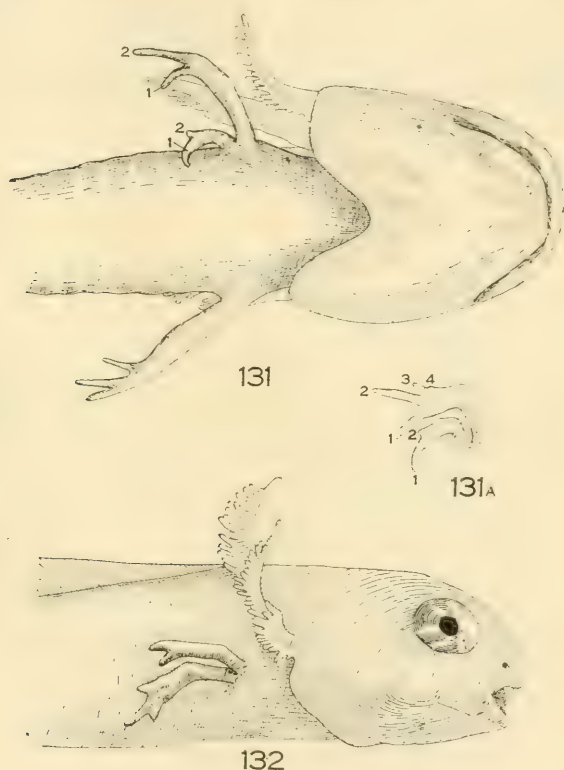
Fig. 131 Transplantation of half limb bud (comb. 10, fig. 120); posterior half left side to anterior right (*het.dd.*). Exp. H. R. E. 9. Anterior member a right (homopleural), the posterior one a left (heteropleural), but imperfect. Ventral view of specimen preserved forty days after operation. × 10.

Fig. 131A Lateral view of limbs of same.

Fig. 132 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half left side to dorsal right (*het.dd.*). Exp. H. R. E. 20. Two right limbs, the anterior one imperfect. × 10.

the proportion of normal results to be as given in table 5, column 6. 'Normal by resorption,' being fundamentally the same as reduplication, is classed as such.
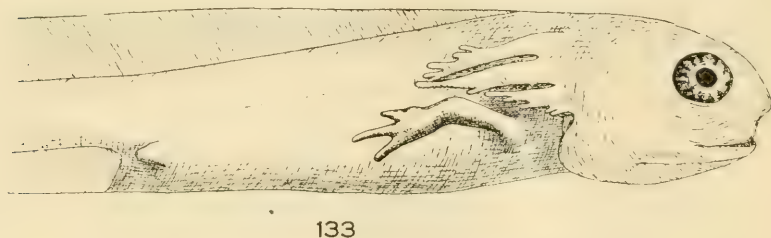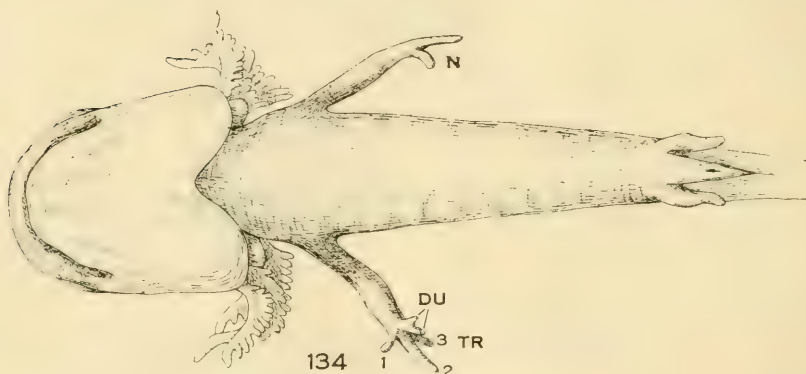
Fig. 133   Transplantations of half limb bud (comb. 16, fig. 120); ventral half left side to dorsal right (*het.dv.*).   Exp. H. R. E. 36.   Normal limb.

Fig. 134   Transplantation of half limb bud (comb. 13, fig. 120); anterior half left side to anterior right (*het.dv.*).   Exp. H. R. E. 10.   *N*, normal left arm; *TR*, grafted arm with palmar reduplication of two digits (*DU*).   × 10.

The one thing that stands out is the great difference between the results of the harmonic and those of the disharmonic combinations.   In the case of none of the other attributes of operation is there anything like the same difference between those of a pair, though in the case of homogeneity vs. heterogeneity the difference is considerable (37.1 vs. 64 per cent).

However, the comparisons cannot be accurately made without the same number of experiments in each class, unless made by means of percentages. This is quite obvious, for instance, in the case of the homopleural transplantations with dorsodorsal orientation, which all result in normal limbs. The relatively

TABLE 5

Transplantation of half buds.   Comparison of pairs of qualities of operation

PAIRS OF QUALITIES COMPARED	NUMBER OF CASES				PER CENT NOR- MAL	PER CENT NORMAL AFTER CORRECTION FOR INEQUAL- ITY IN NUMBER OF EXPERI- MENTS
	Normal	Normal by resorp- tion	Re- dupli- cated[1]	Total		
Homopleural....................	8	4	11	23	34.8	50.0
Heteropleural.....	21	3	13	37	56.8	50.2
Dorsodorsal....................	10	3	10	23	43.5	56.25
Dorsoventral....................	19	4	14	37	51.4	43.9
Homogeneous..................	13	7	15	35	37.1	27.7
Heterogeneous.................	16	0	9	25	64.0	72.5
Vertical........................	14	5	8	27	51.9	53.75
Horizontal.....................	15	2	16	33	45.5	46.8
Anterior or dorsal half trans- planted........................	15	6	11	32	45.45	50.2
Posterior or ventral half trans- planted.......................	14	1	13	28	50.0	50.0
Harmonic (hom. dd and het. dv)	27	0	3	30	90.0	93.9
Disharmonic (hom. dv and het. dd)...........................	2	7	21	30	6.7	6.25

[1] The four cases of anomalous reduplication have been omitted from this tabulation.

small number of cases in this group affects the record for homopleural transplantations by reducing considerably the number of cases that would have developed normally, thereby giving undue weight to the larger number of dorsoventral cases which result in reduplications. Likewise the dorsodorsal vs. dorsoventral record is influenced by the relatively small number of homopleural cases.

On this account the operations in each class have been reduced
to a common basis. While the probable error of these figures is
in most cases large, the comparisons resulting therefrom are
no doubt much more reliable than those resulting from the
figures of the actual experiments. They are given in the last
column of table 5.

In examining this table we find that there is little or no asso-
ciation between the experimental results and the following quali-
ties of operation: homopleural vs. heteropleural, dorsodorsal vs.
dorsoventral, vertical vs. horizontal, anterior and dorsal vs.
posterior and ventral, the deviation from total lack of associa-
tion (50 per cent) being in the most extreme case but 6.1 per cent.
When we examine the figures with reference to the pair, homo-
geneity vs. heterogeneity, we find that there is a much wider dif-
ference (27.7 per cent as compared with 72.5). This would have
to be regarded as a significant difference but, as will be seen below
it is only secondarily so. The marked association between the
harmonic combinations and normal development (93.9 per cent)
and the very small proportion of normal development (6.25 per
cent) in the disharmonic group, show that it is largely this pair
of attributes that determines whether development will be normal
or not. This quality of harmony or disharmony, however, is
not like the simple qualities of side of origin, orientation, or direc-
tion of the incision, but is itself a combination of two of them.
Those that are harmonic are the homopleural dorsodorsal and
the heteropleural dorsoventral combinations, the other two being
disharmonic, as in the experiments with whole limb buds.

When we consider the homogeneous and heterogeneous com-
binations, we find them unevenly distributed with respect to
the harmonic and disharmonic. This is on account of the restric-
tion of operation due to the semicircular shape of the trans-
planted pieces, which makes half of the combinations impossible
of execution. Were these all possible, there would be complete
symmetry in the aggregation as a whole. In reality, it will be
recalled, six of the homogeneous combinations are disharmonic,
while only two are harmonic. On the hypothesis that it is the
harmony of the combination that determines normal develop-

ment, and with an equal number of experiments in all of the six-
teen possible classes, the expectation would be that only 25
per cent of the homogeneous and 75 per cent of the heterogeneous
would be normal. This corresponds closely to the figures 27.7
and 72.9, respectively, found in table 5.

As regards the question of equipotentiality, the results of these
experiments are equally striking. The two homogeneous com-
binations which, according to expectation, should yield normal
limbs did so. Thus two ventral halves yielded normal limbs in
all six experiments, as did two dorsal halves in five out of
seven. In three cases of disharmonic homogeneous combination
normal limbs developed by resorption of the reduplicating bud;
two of these were from two anterior halves and one from two
posterior. Two further cases of normal limbs from two anterior
halves developed without external evidence of resorption. While
the last five, if interpreted according to the rules, can only be
accepted as evidence of equipotentiality in so far as they show
that a whole limb can develop out of a single half bud, the
others show that two half buds which are alike except that they
are from opposite sides of the body may give rise, when harmonic,
to a single normal limb.

GENERAL DISCUSSION

In this section the following questions will be considered:
1) the foundation of the rules of symmetry; 2) the mode of repre-
sentation of symmetric relations in the limb rudiment; 3) the
formation of reduplications; and 4) form regulation and function
in transplanted limbs.

E. The rules of symmetry.

The validity of the rules of symmetry which have already
been stated in the introduction (p. 4) will best be realized by
considering the results of the several experiments in tabular
form (table 6). Conformity is shown most strikingly in the
heterotopic group, where there is only a single apparent excep-
tion in forty-five cases; and this exception, as already pointed
out, is probably due to an error in recording the operation.

In the orthotopic group the lowest percentage of conformity (65.8) is found in the inverted homopleural buds (*hom.dv*), where the exceptions are due entirely to adjustment by rotation of the limb as a whole. In the superposed buds the sole exception is due probably to the same cause. The exceptional

TABLE 6

Showing conformity to rules in the several experiments

OPERATION				RESULTING LIMB				
Type of experiment	Side of origin of graft	Orien- tation	Harmonic or dis- harmonic	Conforming to rules		Exceptions		Total
				Num- ber	Per cent	Num- ber	Per cent	
Whole bud heterotopic..	hom.	dd	harm.	7	100.0	0		7
Whole bud heterotopic..	hom.	dv	disharm.	12	100.0	0		12
Whole bud heterotopic..	het.	dd	disharm.	10	100.0	0		10
Whole bud heterotopic..	het.	dv	harm.	15	93.8	1(?)	6.3(?)	16
Whole bud orthotopic...	hom.	dd	harm.	9	100.0	0		9
Whole bud orthotopic...	hom.	dv	disharm.	25	65.8	13	34.2	38
Whole bud orthotopic...	het.	dd	disharm.	31	100.0	0		31
Whole bud orthotopic...	het.	dv	harm.	16	100.0	0		16
Superposed buds........	hom.	dd	harm.	5	100.0	0		5
Superposed buds.......	hom.	dv	disharm.	4	80.0	1	20.0	5
Superposed buds.......	het.	dd	disharm.	5	100.0	0		5
Superposed buds.......	het.	dv	harm.	5	100.0	0		5
Half buds.............	hom.	dd	harm.	8	100.0	0		8
Half buds.............	hom.	dv	disharm.	15	100.0	0[1]		15
Half buds.............	het.	dd	disharm.	13	86.7	2[1]	13.3	15
Half buds.............	het.	dv	harm.	19	86.4	3	13.6	22
Total...................................				199	90.9	20	9.1	219
Average of percentages.......................					94.5		5.5	

[1] Four cases of anomalous reduplication have been omitted from this tabulation, inasmuch as they cannot be classified either as conforming or as exceptional.

cases arising after transplantation of half buds have already been discussed, and, as pointed out above, there are in this group of experiments obvious disturbing factors which might readily account for the exceptions. Taking the experiments as a whole, 90.9 per cent conform and 9.1 per cent are exceptional, but if allowance is made for the difference in the number of experiments in each class, assuming that each is a fair sample of what would

occur in a large number of cases, then but 5.5 per cent are exceptional.

The behavior of transplanted limb buds in accordance with the above rules indicates that the posture and the asymmetry of the limb is determined neither by the limb itself nor by its surroundings exclusively, but by an interaction between the two. This is best described by the assumption, that in the stages experimented upon the anteroposterior axial differentiation is already determined within the limb bud, while the ventrodorsal axis (probably radio-ulnar of the grown limb) is determined by its orientation with reference to the surrounding tissues of the host (fig. 135). In a given place a right limb bud upside down thus behaves like a left limb bud right side up and vice versa (fig. 2). It is scarcely necessary to point out that this is not a gravity effect, for the embryo lies on its side during the period when the dorsoventral axis of the limb is determined, 'upside down' being used here merely with reference to the cardinal points of the embryo itself.

What the nature of the influence exerted by the organic environment may be, has not been determined. Whether it acts upon the intimate structure of the limb bud or directly upon the differentiating systems contained therein, without affecting the intimate structure as a whole, cannot be answered from the present data (p. 101). The influence is not sharply localized, for it is the same both in the limb region itself and elsewhere along the flank of the embryo, so that it is probably an effect of the axial differentiation of the tissue elements themselves. It is possible that light may be thrown upon this question by transplanting the limb bud to the dorsal or to the ventral midline of the embryo.

F. The mode of representation of symmetric relations in the limb rudiment

The question whether the adult parts are localized in the germ, forming a mosaic, must be answered in the negative for the limb bud, as used in the experiments, i.e., if we consider as such a disc of tissue, three and a half somites in diameter, centering ventral to the fourth myotome, and leave out of account the outlying regions from which certain portions of the shoulder-

girdle develop. This conclusion is based upon the following evidence derived from the experiments: 1) After extirpation of any half of the limb bud, a complete normal limb may develop from the remaining half; 2) fusion of two limb buds by super-position is followed, if the combination is harmonic, by the development of a single normal limb, which at first is usually larger than normal, but in which there is rapid regulation of size; 3)
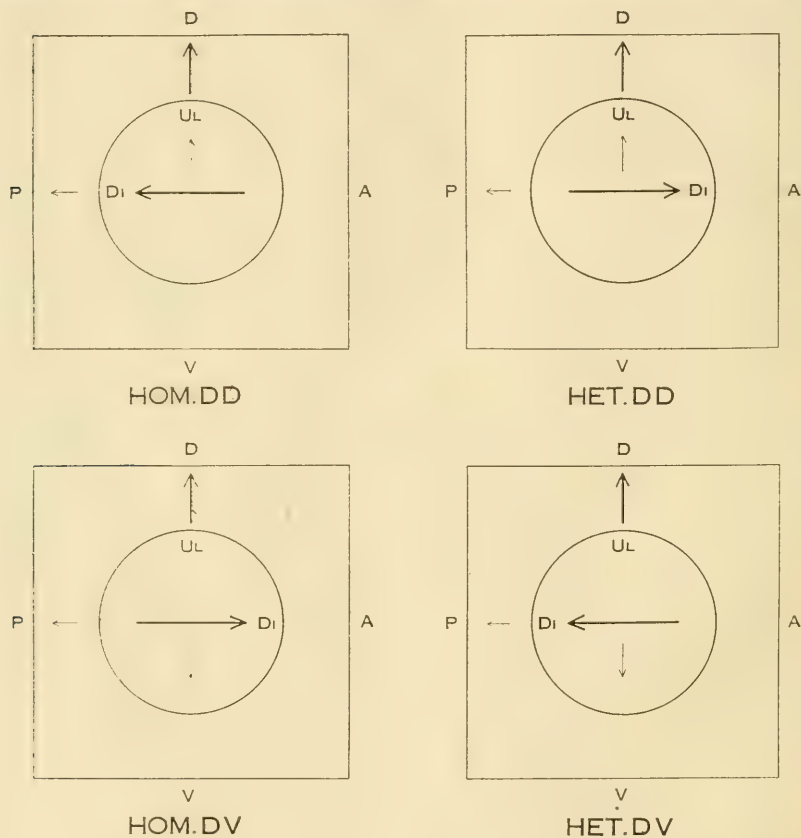
Fig. 135 Diagram to show determination of asymmetry of limb. The circles represent the limb bud, the squares the surrounding part of the embryo. A,D, P,V, the cardinal points of the embryo—anterior, dorsal, posterior, and ventral, respectively. The heavy arrows represent the determining axes, i.e., the antero-posterior axis of the bud and the dorsoventral axis of the surrounding parts; UL, future ulnar border; DI, approximate direction of outgrowth. The smaller arrows show the other axes of bud and surroundings, respectively, which are not effective in determining the axes of the definitive limb.

a normal limb usually develops out of two like halves, i.e., two dorsal, or two ventral halves, if properly oriented, when the opposite half is entirely missing; 4) after inversion of the limb bud the material that normally would have formed the radial half of the limb gives rise to the ulnar half and vice versa, so that practically no part of the bud has the same fate that it would have had if it had been left in place; 5) the inoculation of mesoderm from the limb bud under the skin of the flank of another embryo may result in the formation of a normal limb, although the inoculated tissue is badly disarranged by the operation. According to all tests that have been applied, the embryonic limb rudiment constitutes, therefore, an harmonic equipotential system, though, as a whole, it is self-differentiating except for the determination of its dorsoventral axis. The term 'harmonic equipotential system' is employed here, as defined by Driesch, in the sense that the potencies of all parts of the system are the same, the constituent cells being totipotent.[94] Its use does not imply that the writer attaches to the existence of such systems the same significance as Driesch, who considers them as constituting a proof of the 'autonomy of life.' Even without this, however, and even though the actual system may not reach the abstract perfection demanded by its definition, it remains as a useful conception in experimental morphogenesis. The existence of the equipotential system necessitates, in fact, the assumption of some sort of molecular hypothesis for the representation of adult form in the germ, and herein lies its importance in connection with the present study.[95] In particular, we must look to the constitution of

[94] The concept 'harmonic equipotential system' is defined by Driesch ('05, p. 679) as follows: "Bekanntlich nenne ich harmonisch-äquipotentielle Systeme solche Formganze, bei denen eine Differenzierungs- oder Wachstumsgesamtleistung in ihren Einzelheiten jeweils einzelnen Elementen des Ausgangsganzen zufällt, derart, dass jedes Einzelne dieses Ganzen jedes Einzelne jener Leistung vermag, alles Einzelne aber derart in Harmonie steht, dass die Leistung selbst ein Ganzes ist." The bearing on the question of vitalism is discussed in various papers, especially: '99, p. 99; '01, p. 170; '08 b, p. 138.

[95] Child has expressed skepticism as to the very existence of equipotential systems; for instance: "I think we may say that there is at present no valid evidence for the belief that any living system which is undergoing regulation or development in nature is at any given time an equipotential system" ('11, p. 306). Cf. also Child, '08.

the elementary units of the limb bud, rather than to their arrangement, for the representation of those relations of symmetry that the experiments here described have revealed.[96] In other words, it is the intimate protoplasmic structure that underlies symmetry.

In an equipotential system without axial differentiation, it is most natural to assume that the elements themselves are isotropic.[97] Axial differentiation would then result from the gradual modification of these units by reaction with other elements of the system or through external influences. These differentiations with reference to directions in space may be referred arbitrarily to three axes crossing one another at right angles. They are geometrically of four grades, according to the number of axes along which polarization has taken place.

Taking the models used in stereochemistry to show the spatial relations of the atom groups in certain carbon compounds, we may represent the above four conditions of the elements of the organism or system by four figures (fig. 136) in which the groups that determine the axial relations are situated at the four angles of a tetrahedron. At the center of each tetrahedron we might by analogy assume a carbon atom linked to the four groups occupying the angles of the figure, though this is not necessary for the present purpose. By hypothesis the groups at the angles are supposed to be at first all alike (fig. 136, 1). If one of them should be changed by some reaction, the structure of the molecule would become polarized (fig. 136, 2), and if all the molecules should assume approximately the same orientation, the system which they constitute would show a similar polarity. If two of

[96] The question whether relations of symmetry of the organism are to be based upon symmetrical relations of the intimate protoplasmic structure is answered in the affirmative by Driesch ('08 a, p. 144): "Wir müssen also alle Symmetrie und auch alle Wirkungen, die von äusseren Faktoren ausgehen und sich auf Symmetrie beziehen, auf präformierte, gerichtete Elemente des 'Protoplasmas' beziehen und können in jenen Wirkungen nur richtende und umordnende Geschehnisse sehen.

[97] To avoid misunderstanding, it should be stated that when we speak of equipotentiality and isotropy, we do not lose sight of the fact that the system in its entirety is heterogeneous.

the groups become differently modified, then the structure becomes bilaterally symmetrical (fig. 136, *3*). And, finally, if three become modified, so that all four are different, then the arrangement becomes asymmetrical (fig. 136, *4* and *5*) as in the case of optically active substances with an asymmetric carbon atom. In the last phase there are two kinds of individuals, which are exactly alike in every respect, except that they are the mirror images of
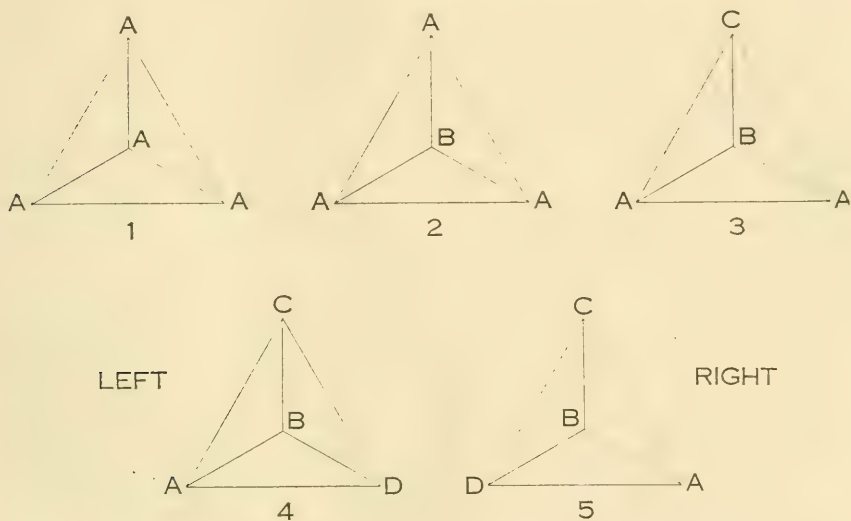
Fig. 136 Diagram to show hypothetical progressive differentiation of the structural units. 1) condition of isotropy; 2) polarization with reference to one axis; 3) bilateral symmetry (two axes differentiated); 4 and 5) condition of complete asymmetry (three axes differentiated) giving right and left enantiomorphs.

one another—in other words, rights and lefts. This is expressed in aggregate form in the right- and left-handed crystals corresponding, respectively, to the dextro- and laevo-rotatory forms of otherwise identical substances.

The experiments with limbs show that the bud at the time of transplantation is in either the second or the third phase, probably the former. There must be a differentiation along the antero-posterior axis, because if this is reversed the limb shows it by growing in a direction nearly opposite the normal. The medio-

lateral axis is probably not differentiated, though in the absence of sufficient experiments in reversal of this axis, it is better to make no definite assumption regarding the intimate structure in relation to it. The dorsoventral axis is at most but slightly differentiated, and if it is at all, then the differentiation is reversible.[98] As already pointed out (p. 55), there is some ground for the latter assumption, for it has been observed that after transplantations in the heteropleural dorsoventral position (the harmonic combination in which the dorsoventral but not the anteroposterior axis is reversed) the adjustment of the tissues of the limb bud is apparently not immediate, but involves a time factor, probably not entirely accounted for by the effect of the operation as such. Whatever the character of this dorsoventral differentiation may be, it is nevertheless very slight in comparison with the anteroposterior differentiation, which has become irreversible by the time the stage in question is reached.

If we could experiment over a wide enough range of stages, it should be possible to determine the time limits of the above phases of axial differentiation of the limb rudiment. At present, however, there are no data bearing upon the question, for in the earliest stages in which the transplantation of limbs has been carried out (embryo with wide open medullary folds), as shown by Detwiler ('18), the limb bud follows in its development the same rules as here formulated.

Lest the foregoing scheme seem too formal, it may be pointed out that the model has been chosen to explain solely the relatively simple characters of polarity and symmetry. Upon this as a basis, further experimentation may yield facts from which the mode of representation of more specific form features may be determined. There is nothing in such a scheme inconsistent with the fact that the cell itself is not a homogeneous system, for the model is supposed to represent only that constituent of the system which determines the adult character in question.

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[98] This is perhaps odd in view of the facts brought out by Przibram ('10 b), showing that dorsoventral differentiation is very marked in the animal organization, more so, for instance, than the anteroposterior differentiation.

The point which it is desired to emphasize is that in an organic 'equipotential system' there must be some intimate structural basis for adult characters in the units that make up the embryonic rudiment.[99] It cannot be in the arrangement of these units; for in that case marked disturbances of development would be produced by such operations as removing half of the rudiment, fusing two buds together, combining two like halves, inverting the dorsoventral axis, or inoculating masses of mesoderm cells from the limb rudiment under the skin of the flank; and yet normal development may follow any of these procedures.

These experiments yield, of course, no information concerning the localization in the cell of the representatives of the adult form characters in question. The system here dealt with is a pluricellular one, but it is interesting to find that in the most thorough and careful studies of polarity and symmetry in the egg, the basis of these properties is found to be in the cytoplasm of the egg cell. Lillie ('06, '09) shows that the polarity of the Chaetopterus egg must be located in the ground-substance, because any amount of shifting of visible granulations in the egg, such as yolk, oil droplets, pigment, etc., has no effect on the polarity of the resulting embryo. With this conclusion the work of Morgan and his collaborators ('08 b, '09, '10) on the centrifuged eggs of various animals, more particularly Arbacia and Cumingia, is in substantial accord. Conklin ('16, '17), in his study of Crepidula, concludes that it is the spongioplasmic framework of the egg-cell that determines its polarity, though he does not consider how this quality is determined in relation to the intimate structure of the spongioplasm. To the extent that Conklin places the seat of polarity in the more viscid rather than in the more fluid constituent of the cytoplasm, he takes issue with Lillie, but in the main, there is agreement between these two investigators. Lillie, however, goes a step further when he says ('09, p. 77): "The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when

[99] Cf. Driesch, l. c.

the yolk granules are packed closely into the small cell of the two-celled stage of Chaetopterus) seem to me to argue for a molecular basis of the fundamental principle of vital organization." Morgan, likewise, takes this view when he says ('09, p. 114), "These considerations incline us to the view that there exist in the molecular constitution of the egg the potential factors of symmetry." The scheme outlined above is in harmony with this concept.

On the other hand, Child ('13, '15), as also Della Valle ('13) rejects all such hypotheses, basing the phenomena of axial differentiation upon the occurrence of gradients, which, according to Child, are primarily of a functional (metabolic) nature. It seems to the present writer that such gradients may well be an expression of the polarity rather than its cause.

G. Reduplication and the problem of polarity and heteromorphosis

The reduplications which have been observed in the various experiments have already been described sufficiently for the present discussion (pp. 35, 45, 65, 73). The salient facts are: 1) that the duplicate is the mirror image of the original limb; 2) that more than one secondary member may arise by budding from the same primary bud, in which case both of the former stand in some relation of symmetry to the original; 3) that the secondary appendages themselves may be doubled, forming a more or less symmetrical pair. There are a few exceptional cases, where two members of the same side stand in linear series, but probably these have arisen only where the two rudiments are far enough apart not to influence one another.[100]

[100] Several cases are to be considered here. One (H. R. E. 10-) is a case in which the anterior half of the limb bud was removed. Two limbs developed, one clearly from the remaining posterior half and the other probably from the anterior border of the wound (cf. Harrison '18, p. 441). The operation was done on the left side and both limbs were lefts, the posterior one being somewhat defective. In another case, which had an early history similar to the above, the posterior member was very defective and it was impossible to determine whether it was a right or a left. A third case (H. R. E. 20) is the one figured on page 79. This is not a case of regeneration, but one in which the anterior member probably developed from the grafted half, while the posterior member may have developed from the stationary ventral half. Both are attached to the same shoulder-girdle, but there are two separate glenoid cavities.

Bateson, in his "Materials for the Study of Variation" ('94), has given an exhaustive review of the literature relating to supernumerary parts, in which the limbs are fully considered. In this treatise he has made a masterly analysis of the available material, particularly with reference to the appendages of arthropods. The phase of the problem which is especially relevant to the present discussion is that concerning what Bateson calls minor symmetries, in which the supernumeraries are in some way symmetrical with respect to themselves or to the normal appendages with which they are associated. The other class of supernumeraries, in which two identical appendages stand in simple succession to one another are, according to Bateson, practically unknown, and even those that have been described are considered by him to be of somewhat doubtful nature, though many cases of simple hyperdactyly would seem to belong in this category.

The symmetrical extra appendages fall into two groups: 1) those in which there is a pair of extra members symmetrical with themselves, arising from the normal appendage with which one of the supernumeraries appears to have a definite relation of symmetry, and, 2) those in which the single supernumerary is symmetrical with respect to the normal. The former condition Bateson considers to be the more usual, and, in fact, he accepts the existence of the latter with a certain skepticism which seems unnecessary.[101] It is true that many cases that apparently fall within the latter group may upon closer examination be found to belong in the former, but the converse is also true, as will be shown below (p. 97). Bateson has devoted special attention to the first group, and, on the basis of about one hundred and twenty cases in insects and a considerable number in the Crustacea, he has formulated the following rules,[102] showing the relation of the supernumerary appendages to each other and to the original member:

I. The long axes of the normal appendage and of the two extra appendages are in one plane: of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other is remoter from it.

[101] Op. cit. p. 539 and 553. Consider, however, in this connection, the clear case described by Bender ('06).

[102] Op. cit. p. 479.

II. The nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

Transverse sections of the three appendages taken at homologous points are thus images of each other in parallel mirrors.

In the vertebrates Bateson marshals a large amount of material, of which about fifty cases are in amphibians.[103]   At the time Bateson's book was written, however, little or nothing was known regarding the origin of supernumera y appendages in either the arthropods or the vertebrates.   Since then a large amount of experimental evidence has accumulated to show that they may be formed by superregeneration, especially by regeneration from complex or irregular wound surfaces.[104]   The evidence all corroborates Bateson's main gene. alization regarding the relation of symmetry of supernumerary limbs, and there are practically no exceptions.[105]

The importance of double supernumeraries (Bruchdreifachbildung, la doppia rigenerazione inversa, see p. 95) is emphasized in the papers by Emmel ('07) and Della Valle ('13), and this conception is given prominence in Przibram's more general discussion of the question ('09, p. 234).

[103] See Bateson (pp. 554–5) for a discussion of the older literature.

[104] In the amphibians the investigations of Barfurth ('94), Giard ('95), Tornier ('97, '00, '05, '06), Lissitzky ('10), Fritsch ('11), Kurz ('12), Della Valle ('13), and others have added much to our knowledge of the subject.   In the crustaceans Przibram ('02), Reed ('04), Zeleny ('05), and Emmel ('07) have reported experiments which, though not so numerous, are none the less important.   The more recent literature is fully discussed in many of these papers, especially in those of Lissitzky, Fritsch, and Della Valle, to which the reader is referred for details.

[105] A remarkable exceptional case has recently been described by Dawson ('20) in a lobster, in which there is an extra pair of chelipeds attached to the normal.   The two extra chelae are mirror images of one another, but the one nearer the primary claw is not mirrored from the latter, but is of the same side. Furthermore, the primary claw is a 'nipper,' while the supernumeraries are both of the 'crusher' type, so that the case proves to be likewise an exception to Przibram's rule ('11), according to which, in heterochelous forms, the extra appendages are of the same type as the primary.   The case described by Cole ('10), also in a lobster, is an almost diagrammatic example of Bateson's rule, if allowance is made for the effects of torsion.

Likewise, the well-known experiments of Tornier ('05) upon tadpoles of Pelobates, in which the hind-limb bud was divided in an early stage, some of the cases of Lissitzky ('10), and Della Valle's case of reversed regeneration conform closely to Bateson's rules. Although the end results of the experiments of Tornier and of Della Valle are analogous, there is, however a sharp difference of opinion regarding the exact mode of origin of Tornier's double supernumerary hind legs, Tornier maintaining that they both arise from the dorsal part of the pelvis, which was split off by the operation, while Della Valle holds them to be analogous to his own case.

Della Valle has laid particular stress upon the supposed identity of change of asymmetry and reversal of polarity, and has sought to make the various cases of superregeneration which have been reported fit into his scheme of 'doppia rigenerazione inversa.' The case on which Della Valle bases his discussion of these questions is that of a newt (Triton) in which the left anterior limb was fractured in the region of the brachium and cicatrization prevented by tying a silk thread around the limb at that level. Twenty days later the same limb was amputated a short distance below the point of fracture. There regenerated three perfect limbs, one from the distal end of the stump and two from the region of the fracture. Of the latter, one was from the proximal end of the small portion beyond the ligature and the other was apparently a continuation of the stump proximal to the fracture. The first and last of the three were left limbs, i.e., of the same side as the original, while the one which regenerated in a distoproximal direction was reversed. The end result was a triple appendage in which the three members were placed in accordance with Bateson's rule.

Della Valle seeks to make the cases of Tornier ('05) and Lissitzky ('10) conform to this scheme, and falls into line with Przibram ('09) who had previously given a schematic representation of the same phenomena, which he termed 'Bruchdreifach-bildung.' He also interprets in the same way the reduplications obtained by Braus ('04, '09) and myself ('07) in the transplantation of embryonic limb buds. He suggests that when the limb

bud is implanted in normal location, triplicate appendages could be accounted for in the following way: one member derived directly from the grafted bud; one member, of mirror symmetry, by an inverse regeneration from the base of the bud, and the third member, of original asymmetry, from the wound surface of the host. This view is, however, not borne out by the present experiments.[106]

When the whole of the evidence bearing on the question is taken into consideration, one cannot but think that too much weight has been placed by Bateson and his followers on the double supernumerary. The other class of cases, where the single supernumerary is symmetrical with the normal appendage with which it is associated, while neither so numerous nor so spectacular, is nevertheless of wide occurrence. Cases reported by Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), and Megušar ('07) show that truly double appendages in mirror symmetry with respect to each other may be formed by constric-

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[106] Se dunque noi considerassimo uno di questi innesti praticati invece che in una regione lontana (come p. es. nell'orbita), nell'immediata vicinanza della regione donde fu tolto l'innesto, noi osserveremmo l'uno presso dell'altro lo sviluppo oltre che dell'arto normale, anche dell'arto rigenerato dalla superficie di sezione della regione prossimale del corpo, nonchè dell'arto sviluppatosi dalla superficie di sezione della regione periferica, identico all'arto che lo ha prodotto, ma con simmetria speculare. La identità anche di questo fenomeno con la doppia rigenerazione inversa dalle due superficie di una ferita risulta in questo modo evidente. Della Valle: op. cit. p. 125.

There is opportunity to test this hypothesis by comparing the experiments in which the wound-bed was cleaned with those where it was not. In the former, regeneration from the host is precluded (p. 6), and triplicate limbs could only arise by a second reduplication from the base of the graft; whereas in the latter, regeneration from the host should occur in a large number of cases, if at all, and thus yield a large proportion of triplicate appendages. An examination of the results shows that this is not the case. In the first place, as shown in table 2, the total number of reduplications in the series with cleaned wounds is fifty-three, which is 56 per cent of the total number of positive experiments, while there are but sixteen cases (33.3 per cent) in the group with non-cleaned wounds. The disproportion is much greater when the number of triplicate appendages in each group is compared. Out of a total of eighty-seven cases old enough to be determined, there are twenty-five triplicate limbs (28.7 per cent) in the clean-wound experiments and only three in forty-eight cases (6.25 per cent) in the others. It is quite clear, then, that leaving in the wound-bed cells that are capable of giving rise to a new limb reduces greatly, instead of increasing, the chance of formation of supernumerary limbs, so that Della Valle's suggestion is untenable.

tion of a simple regenerating bud. This harmonizes with Driesch's ('06) observations on double Echinus embryos.

In the present work, the reduplicated extremities are nearly all found to be in minor symmetry, and many of those in which three members are present, if seen only in the fully developed condition, would appear to be cases of paired supernumeraries, conforming, though with some aberration, to Bateson's rules. The individual histories show, however, that they are mostly simple duplicities in which the supernumerary mirrors the original, and this seems to be the case in Braus's experiments, too. Two reduplicating limbs often do develop, but usually each grows as a bud from the original instead of the two arising as a pair in themselves. Each of them mirrors the original limb, so that the two supernumeraries are both of the same side. In other cases the supernumeraries are themselves double, in which event there is strict conformity to Bateson's rule, but the former constitute a large majority, and conformity there is only superficial, for the original limb is the middle member and not one of the extremes.

In view of these facts, there is probably no very fundamental difference between the two classes of reduplications, i.e., between the double supernumeraries symmetrical with each other and the single supernumerary symmetrical with the original; had Bateson had the developmental stages at his disposal, he himself might not have drawn so sharp a distinction.

In accordance with the above, Bateson's rules might be stated in more general form, so as to include both simple duplicities and symmetrical pairs, as follows:

1. The long axes of duplex or multiplex appendages lie in one plane.

2. Two adjacent members form in structure and position the image of each other, as reflected from a plane mirror bisecting the angle between the respective axes and perpendicular to the common plane of the two axes (figs. 3 and 4).

The present experiments show (tables 2, 3, 5, and 8) that, excepting heterotopic grafts, it is in the disharmonic combinations that reduplications are most frequent. What, now, is the nature of the disturbance that causes the doubling of transplanted

limb buds and of regenerating limbs, which, when it occurs, is
always combined with reversal of one member? The first visible
sign of reduplication both in the embryonic limbs and in the re-
generating blastema is the presence of two growth centers for the
limb in place of one; each becomes an apex of growth, with a
resulting bifurcation of the appendage as a whole. The question
arises whether the doubling of the growth center is antecedent
to or resultant from the reversal of the asymmetry. From the
fact that mere mechanical division of a simple regenerating cen-
ter[107] may bring about doubling, it would seem to be more prob-
able, if not certain, that the existence of two growth centers
within spheres of mutual influence is the factor that produces
the reversal in one—the one that is less advantageously placed,
or in which differentiation is less advanced.

The problem before us thus resolves itself into two phases:
that of division or repetition of parts and that of symmetry.
This was clearly seen by Bateson, who has emphasized the funda-
mental nature of the power to divide.[108] No attempt will be
made here to analyze this phase of the question. The symmetric
relations of the repeated parts are, however, so definite and of
such general recurrence that they, too, are beyond question of a
fundamental nature.

The phenomenon of reversal of asymmetry has been treated
by many investigators as one with that of axial heteromorphosis,
and yet this is not strictly correct, for the reversal of asymmetry
may be brought about by the interchange of the poles of any one
of the three axes to which the object is referred, and not neces-
sarily the one along which regeneration and differentiation is
taking place. This is true not only when regeneration occurs in
a proximodistal direction, as in the cases of Tornier, Zeleny, and
others, cited above, but also when it takes place distoproximally,
as shown in the two experiments reported by Kurz ('12).[109]

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[107] Cf. Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), Megušar ('07).

[108] "This power to divide is a fundamental attribute of life and of that power
cell division is a special example." (Problems of Genetics, p. 38.)

[109] In somewhat similar experiments by Morgan ('08 a) only the bone, not the
soft parts, was reversed. Nothing is said regarding the exact character of the
limbs regenerated.

The fundamental phenomenon, therefore, is not that a particular axis is reversed, but that reversal occurs at all, and how it is brought about.

Organic polarity, in general, has been based either on the supposed polarization of the organic units themselves or upon a supposed gradient of a functional (Child) or material (Morgan, '05) nature, running from one end of the organism to the other. There is evidence for the occurrence of both factors, and what seems most likely is that both are at play. Under certain circumstances they act in the same direction; under different conditions one may antagonize and retard, or even overcome, the other, as seems likely in heteromorphic regeneration where polarity is reversed (earthworm, planarians, amphibian tail, etc.). Przibram ('13), who advocates a theory combining the two factors, which he calls 'Richtungspolarität' and 'Schichtungspolarität,' respectively, nevertheless regards the reversal of polarity as due to actual rotation of the cells. He ('06, '10 a) cites unpublished work by Hadži in support of this view, and adopts it in his diagrams ('09) illustrating the five fundamental varieties of operation leading to regeneration (Biotechnik).

The figures are not convincing, however, for just as much rotation of the cells is shown at the end where polarity is not reversed as at the other end where it is reversed (Przibram, '09, pl. XV, 1h 3h), and in fact, as expressed in these diagrams, what turning is shown is nothing more than a wound-healing process. Until it is demonstrated that rotation of the cells as a whole takes place solely in heteromorphic regeneration, it cannot be used to explain reversal of polarity.

So long as the elementary units of the limb bud have one plane of symmetry left, and the final asymmetry of the limb remains to be determined by its relation to certain axes of the embryo,[110] it

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[110] In the case of asymmetric organisms, the elementary units, if representing the form of the organism at all, must be postulated as asymmetric themselves. In the case of paired organs, each asymmetric in itself, but symmetrical with respect to its opposite, polarization on the transverse axis may be assumed as due to the position of the parts with respect to the other two axes (cf. Przibram, '13, p. 38) and not as necessarily due to actual differentiation of the elements in the transverse direction.

will of course be possible to account for its reversal by rotation of
the elements about the proper axis. As an alternative to the
rotation hypothesis, we might, however, consider reversal as due
to an interchange in position of two of the determining groups in
the elementary units (p. 89, fig. 136). In case of differentiation
on all three of the axes, i.e., if the units themselves are asymmet-
ric, then reversal could take place only in the latter way, unless
it occurs altogether independently of the intimate structure.

There is an analogy for reversal of this kind in the change of
the asymmetry of organic molecules of known composition, as,
for instance, in the Walden inversion by means of successive sub-
stitutions, or in the conversion of dextrotartaric acid into racemic
acid, by which transformation half of the dextrorotating groups
are changed into the laevo form. Of course, these examples are
mere analogies.

Such questions have been touched upon by many of those who
have studied twins and double monsters, but, unfortunately,
the evidence both as to the cause and as to the occurrence of
reversal of asymmetry is conflicting. In the case of human dupli-
cate twins, it is certain that there is no situs inversus viscerum,
except very rarely, and apparently even in double monsters the
degree of fusion of the two individuals must be considerable for
the asymmetry of the internal organs—heart and alimentary
canal—to be reversed. On the other hand, it has been shown
by Wilder ('04) that in duplicate twins the friction-skin patterns
of the two mates may show mirror imaging, particularly those
on the index fingers. A similar condition has been found by
Newman ('16) in his study of variation of the scutes in armadillo
quadruplets, except that here the matter is further complicated
by the relation, in pairs, of the four individuals of a litter.[111]

[111] "Now in the armadillo there are many definite evidences of a system of
symmetry common to all of the quadruplets, upon which has been superimposed
a secondary symmetry system between twins. This in turn is more or less com-
pletely obliterated later by a tertiary symmetry between the antimeric halves of
the single individuals. In some sets evident traces of the primary system of
symmetry persist as mirror-image relations between individuals of opposite
pairs, but it is more usual to find no trace of the primary system. The secondary
mirror-imaging between pairs is far more commonly in evidence, but is frequently

The evidence which Morrill ('19) has collected from the study of double monsters in fish embryos shows that situs inversus does occur, but that it is the exception, not the rule, and that there is no "very precise relation between the amount of separation of the two components and the occurrence of mirror imaging."[112] This would seem to oppose the view expressed above, that it is the proximity of two growth centers that causes the reversal of one. Still, in the absence of statistical data regarding the correlation of the two events, it is unsafe to draw a definite conclusion.

In this connection the recent work of Spemann and Falkenburg ('19) is of the greatest importance. By extension and modification of the earlier methods of the former, these investigators obtained a large number of twins in Triton by constricting the eggs in segmentation stages or in the early blastula. They found that in a large number of the cases one individual of a pair (the right-hand member in all cases but one) has complete situs inversus viscerum. Spemann, after an admirable critical analysis of the question, reaches the conclusion, that while some asymmetric intimate structure must be postulated to account for the normal asymmetry of the vertebrate body, there is no proof from these

obliterated by the tertiary mirror-imaging between antimeric halves of the same individual, which latter is the prevailing symmetry system. . . . . In general, mirror-imaging between individuals of opposite pairs is interpreted as an evidence of the early system of symmetry present in the embryonic vesicle before polyembryonic budding began. When the primary buds are formed they are the product of the antimeric halves of the undivided embryo and therefore should have mirror-image relations, but a partial physiological isolation of the two buds permits a certain degree of reorganization or regulation in the symmetry relations, that tends partially to obliterate the original symmetry relations of the undivided embryo. Similarly, when each primary bud subdivides to form the secondary buds that are the primordia of the definitive individuals, a certain residuum of the primary bud symmetry system is carried over, manifesting itself in mirror-imaging between the twins derived from the same primary bud. But here again a certain amount of regulation occurs so that a third system of symmetry, the bilateral symmetry of each individual, tends to obliterate former systems of symmetry." Newmann: op. cit., pp. 200–201.

[112] Tannreuther ('19, p. 359) has recently figured a double chick embryo in which the two individuals are united only by the posterior tip of the primitive streak. Although trunk and head are entirely separate, the heart of the embryo on the left shows situs inversus. No mention is made of this fact in the text.

experiments that it has been completely reversed by the opera-
tion. The evidence points, on the contrary, to the introduction
of another factor which is manifested also in the tendency of the
individuals to show defects on the inner side (i.e., the side turned
toward the partner). In the case of the left-hand member,
this acts in the same sense as the innate tendency to asymmetry
of the viscera, while in the case of the right-hand member, it
antagonizes, and in many cases, overcomes the latter.[113] Spe-
mann suggests many different experiments to throw light upon
this question. The interesting point in Spemann's discussion,
in connection with the present work, is that the necessity for as-
suming some sort of intimate structure to account for external
symmetry relations is recognized. In the case of the redupli-
cated limbs it is not clear whether the reversal of the secondary
bud is a result of direct action upon the individual processes of
development going on within it, or whether the influence of the
primary bud actually reverses the intimate structure. If it
should be found that the reaction takes place before the cells of
the limb blastema lose their totipotence, then the latter is
undoubtedly true. Otherwise it may be that the differentiation
in the limb blastema takes place directly under the influence of
the tissues of the environment.

H. Form regulation and function in transplanted limbs

That the limb bud after transplantation becomes adapted in a
measure to the new conditions is obvious from a casual con-
sideration of the experimental results. There are different types
of adaptation, however, for although regulation of form and func-
tion go largely hand in hand, in some cases there may be very
complete functional regulation without form regulation,[114] and,
particularly in the heterotopic grafts, form regulation without
function. By form regulation is meant, in the present connec-
tion, the process by which a limb bud that is implanted abnor-

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[113] Cf. also Pressler ('11).

[114] For instance, in Experiment I. E. 64, the single disharmonic limb func-
tioned very actively and effectively.

mally in relation to the embryo as a whole becomes adjusted so as to give rise to a limb in harmony with its new surroundings. Perfect adaptation is attained, however, only in cases where there is functional adaptation as well, i.e., only when both form and function are adjusted to the organism as a whole, and this occurs only in the orthotopic position or in positions very close to it. Functional regulation is here largely a question of innervation, and, inasmuch as this has been made a subject of special investigation by Detwiler ('19, '20), it will not be considered at present further than is necessary in its relation to form regulation.

Considering only the orthotopic grafts, there is no a priori ground for expecting any of the combinations to yield normally functional limbs except the homopleural dorsodorsal group, in which the axial relations of the transplanted bud remain normal with respect to the cardinal points of the embryo. Nevertheless, it has been found that inverted limb buds from the opposite side of the body yield almost as high a proportion of normally formed and normally functional limbs as the first-named group. This phenomenon has been interpreted as due not so much to a secondary regulation as to the structure of the elementary units of the limb bud, which are supposed to be symmetrical or reversibly differentiated (p. 89) along their dorsoventral axis. With an equal number of experiments in each of the four groups and without any disturbing factors, the primary processes of regulation should therefore lead, in accordance with the fundamental rules of symmetry, just as often to adaptive (harmonic) as to non-adaptive (disharmonic) end results, but not more often. This proportion is, however, exceeded by nearly 20 per cent of the expectancy, so that, taking the orthotopic experiments as a whole, 59.6 per cent yielded normally functioning limbs in normal posture, while 40.4, for the most part reduplications, were non-adaptive or only imperfectly adaptive (table 7). The proportion is almost the same in the three classes of experiments, whether with whole buds after extirpation of the normal bud, with superposed buds, or with half-buds, though, as more fully discussed below (p. 107), it is quite different in the heterotopic grafts.

TABLE 7

Showing the adaptive or non-adaptive character of the resulting limbs after orthotopic transplantation

TYPE OF EXPERIMENT	TOTAL NUMBER	ADAPTIVE (SINGLE HARMONIC LIMBS)				NON-ADAPTIVE (REDUPLICATION OR SINGLE DISHARMONIC LIMBS)			
		Primarily	By secondary regulation	Total	Per cent	Primarily	By secondary regulation	Total	Per cent
Whole buds									
hom. dd	9	9	0	9	100.0	0	0	0	0.0
hom. dv	38	0	10	10	26.3	25	3	28	73.7
het. dd	31	0	5	5	16.1	26	0	26	83.9
het. dv	16	15	0	15	93.8	0	1	1	6.3
Total	94	24	15	39	41.5	51	4	55	58.5
Average of percentages. . . .					59.0				41.0
Superposed buds									
hom. dd	5	5	0	5	100.0	0	0	0	0.0
hom. dv	5	0	1	1	20.0	4	0	4	80.0
het. dd	5	0	1	1	20.0	4	0	4	80.0
het. dv	5	5	0	5	100.0	0	0	0	0.0
Total	20	10	2	12	60.0	8	0	8	40.0
Average of percentages. . . .					60.0				40.0
Half-buds									
hom. dd	8	8	0	8	100.0	0	0	0	0.0
hom. dv	17	0	4	4	23.5	13	0	13	76.5
het. dd	17	2	3	5	29.4	12	0	12	70.6
het. dv	22	19	0	19	86.4	0	3	3	13.6
Total	64	29	7	36	56.3		3	28	43.8
Average of percentages. . . .					59.8				40.2
Total harmonic	65	61	0	61	93.9	0	4	4	6.1
Total disharmonic	113	2	24	26	23.0	87	0	87	77.0
Grand total	178	63	24	87	48.9	87	4	91	51.1
Average of percentages[1]					59.6				40.4

[1] As given in the three main groups in the upper part of the table.

The secondary regulation of form may be brought about in one of two ways: either by rotation of the limb as a whole during development, whereby it is gradually brought into normal posture, or by a process of reduplication, which is more complicated. In the latter the original grafted limb bud gives rise to a secondary or reduplicating bud of mirror symmetry, which outstrips the former in development, reducing it to a spur or even practically suppressing it. The process of reduplication more often yields, however, actual double appendages, and there is no hard and fast line between the latter and the single limbs with the original bud reduced to a spur. The figures given in the table are therefore somewhat arbitrary in this respect.

Regulation by rotation has been noted only in the inverted limb buds from the same side of the body (*hom.dv*), and it has been further shown that this mode of adjustment probably occurs when the limb bud at the time of operation is rotated anteriorly along the dorsal semicircumference somewhat less than 180° (p. 41). The mechanics of this process is not yet understood. In the other disharmonic group single limbs are very rare (only one case), unless reversal by reduplication and reduction occurs, and no cases of rotation have been observed.

Regulation by reduplication and reduction, which is attended by reversal of asymmetry, has been observed in five cases out of thirty-one in the heteropleural dorsodorsal group, but no perfect cases of single limbs so produced have been found in the other disharmonic combination (*hom.dv.*). In the latter group, however, reduplication is frequent, and the reduplicating member is often normally attached and assumes the same posture as the normal limb, but it has never been found to begin its development early enough to bring about the suppression of the original bud.

In a measure offsetting the regulative cases, there are four others in which the same process, reduplication, has resulted not in regulation, but rather in its prevention. In three cases of inverted buds[115] (*hom.dv.*), where there was tendency to regulate by rotation, this regulative process was rendered futile by secondary budding, and also in one case of a heteropleural dorso-

[115] I. E. 86, 88, 90 and possibly two other cases. See p. 37.

ventral graft, the usual primary regulative result was vitiated by
reduplication. The same was true in three cases of half-buds
inverted on the opposite side of the body (*het.dv.*). Reduplica-
tion, therefore, is not in itself a regulatory process, but leads
merely to a condition where regulation may take place through
the reduction of the disharmonic member.

The question now arises, whether the experiments give any
ground for assuming that there is anything teleological in these
regulatory processes. While there is no proof that regulation by
rotation is not of this nature, it would be a mistake to draw any
conclusion regarding it until the details of the process are better
understood. With regard to regulation through reduplication
and reduction, it is clear that mechanical factors are sufficient
to account for the process. It is in the disharmonic combinations
that secondary regulation is necessary to produce normal results.
When this is not accomplished by rotation, reduplications almost
always arise, and there are only two cases of single disharmonic
limbs among the orthotopic grafts. The disharmonic relation,
which is brought about by the inversion of the anteroposterior
axis of the limb bud, is thus seen to be a factor of prime import-
ance in the production of reduplications. It is not the only
factor, however, for some reduplications have occurred in har-
monic combinations, due probably to other disturbances at the
time of operation. But quite aside from the latter, it would be
wholly unjustifiable to assume that there is any causal relation
between the possible utility of the process of reduplication for
regulatory purposes and its frequent, or even almost universal,
occurrence where it might lead to this result. What seems to be
the condition that brings about regulation after the reduplicating
bud has arisen is the chance placement of the latter in a position
corresponding to that of the normal single limb. This relation
is attended by a more advantageous situation with respect to
blood and nerve supply than that occupied by the original bud,
and in extreme cases this leads to the resorption or suppression
of the latter. It is only in such cases that complete regulation
takes place and these constitute but about 15 per cent of the
total number of reduplications.

In this connection it is important to compare the orthotopic with the heterotopic operations in respect to the regulatory processes just considered. When the limb bud is implanted in abnormal location, functional adaptation does not occur at all, the limbs rarely showing any motor function whatever, unless placed close to the normal position of the limb.[116]

Now the records show (table 8) that the two harmonic combinations in the heterotopic series produced ten single harmonic limbs and twelve reduplications (44.4 and 55.6 per cent, respectively).

TABLE 8

Comparison of heterotopic and orthotopic transplantations with reference to the relative number of single limbs and duplicities in the harmonic and disharmonic combinations

CHARACTER OF COMBINATION	HETEROTOPIC				ORTHOTOPIC			
	Single		Reduplicated		Single		Reduplicated	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Harmonic...............	10[1]	44.4	12	55.6	24	96.0	1	4.0
Disharmonic............	19	86.4	3	13.6	2[2]	3.4	57[3]	96.6

[1] Excluding one anomalous case in which an error of record is probable.

[2] Excluding five cases which became normal single limbs by development of the duplicate and resorption of the original bud, and ten cases which became normal by rotation.

[3] Including five cases in which the original member was resorbed and the single normal limb arose from the reduplicating bud.

In many of the latter, however, the doubling was but slight, involving only the digits. The two disharmonic combinations, on the other hand, produced nineteen single limbs (86.4 per cent) and three (13.6 per cent) reduplications. The results obtained by Detwiler ('18), using much younger limb buds from embryos with open medullary folds, sustain the above results, as far as the harmonic group is concerned. There are eight cases of single limbs and ten reduplications. In the disharmonic group, how-

[116] This has been subjected to a careful analysis by Detwiler ('19, '20), who has shown that the failure to function is not due to lack of peripheral innervation so much as to the insufficient connections within the central nervous system.

ever, there are four normal limbs and three reduplications. If combined with the present results, this would reduce the disproportion somewhat, though it would still leave it very large. There are no cases of complete regulation over to the harmonic position either by rotation or reduplication. Moreover, reduplication in over half the harmonic grafts disturbs the possible harmonic end result.

As pointed out above, the case is very different in the orthotopic operations. Here the harmonic group produced twenty-four out of twenty-five (96 per cent) single limbs, while the disharmonic group produced but twelve single limbs (17.4 per cent), ten of which became harmonic by rotation and are therefore omitted from the tabulation, and fifty-seven reduplications (82.6 per cent), in three of which, however, the primary bud became harmonic by rotation and a possible adaptive result was prevented by the process of reduplication. Leaving out of consideration the cases in which single normal limbs were produced by rotation, there are but two cases left (3.4 per cent) in which the disharmonic relation failed to be followed by reduplication.

In a word, with orthotopic grafts the almost completely dominant factor in producing single limbs or reduplications is the harmony or disharmony of the combination, whereas the case is quite different in the heterotopic grafts, where the harmonic group produced a slight preponderance of reduplications and the disharmonic group a great preponderance of single limbs.

At first sight the above figures might be advanced in support of some hypothesis of an end purpose in development, inasmuch as reduplications are produced in overwhelmingly great number only where they may be taken advantage of to produce an adaptive end result. This, however, would be a hasty conclusion to draw. The orthotopic experiments may be explained as above (p. 106). There the tendency to reduplicate is due first to the disturbance of operation, which, being very slight, is almost always suppressed in the harmonic combinations by the advantage the primary bud has in connecting normally with the surroundings; while in the disharmonic combinations the tendency to reduplicate is not only greatly increased by the reversal of the axis

of the bud, but the primary bud also gains a headway in development that usually cannot be overcome even by the more advantageous position of the reduplicating bud. In the heterotopic operations the frequency of reduplication in the harmonic group may be ascribed to the disturbance due to the operation, together with lack of any special anatomical relations at the seat of implantation that would overcome the tendency to bud by giving the harmonic member a special advantage. All that remains to be accounted for is, therefore, the small proportion of reduplications in the disharmonic group. This should be subjected to further investigation. Standing alone, it can hardly be advanced as an argument for a teleological theory of development.

GENERAL SUMMARY

1. The results given below are based upon the following experiments with the fore-limb bud of the embryo of Amblystoma punctatum:

   a. Transplantation to the flank of another embryo posterior to the normal position of the fore limb (heterotopic transplantation), the grafted buds being taken either from the same side of the body (homopleural) or from the opposite (heteropleural), and implanted with the dorsoventral axis upright (dorsodorsal) or inverted (dorsoventral).

   b. Transplantation to the normal location of the fore limb after extirpation of the original fore-limb rudiment (orthotopic transplantation), with the same variations as in the heterotopic group.

   c. Superposition of one limb bud upon another after removal of the ectodermal covering of the latter, also with the same variations as in the previous groups.

   d. Transplantation of half of the circular disc constituting the limb bud, after extirpation of one-half the rudiment. Sixteen different combinations of this experiment (all possible within the limitations imposed) were tried (p. 70).

2. In the early stages of development in any position the transplanted buds give evidence of their constitution by growing out

('pointing') in the direction of what was originally the posterior
pole of the anteroposterior axis. Thus, in two of the combina-
tions (homopleural dorsoventral and heteropleural dordosorsal)
they point anteriorly or dorsoanteriorly, and in the two others
(homopleural dorsodorsal and heteropleural dorsoventral) they
point posteriorly or dorsoposteriorly like the normal. In the
latter case the subsequent development is usually normal, bar-
ring reduplication; in the latter there is a tendency for the limb to
stick out to the side and to rotate more or less from the position
in which it would be found, were the position determined entirely
by the orientation of the bud itself.

3. The palmar surface of the limb tends to develop on the side
turned toward the body of the animal, and the ulnar border is
dorsal, although the rotation mentioned in the previous para-
graph tends to change these positions.

4. The above circumstances determine the asymmetry of the
limb as follows: when the dorsoventral axis is not inverted, the
original prospective asymmetry persists; when the axis is in-
verted, the asymmetry is reversed (rules 1 and 2, p. 4). In more
general terms: the asymmetry of the limb is determined by two
factors, the polarization of the anteroposterior axis of the limb
bud and the orientation of the limb bud with respect to the dorso-
ventral polarization of its organic environment (figs. 2 and 135).

5. In two of the combinations (homopleural dorsodorsal and
heteropleural dorsoventral) the asymmetry of the limb which
develops corresponds to that of the side of the body on which it is
placed (harmonic); in the other two (homopleural dorsoventral
and heteropleural dorsodorsal) it corresponds to that of the oppo-
site side (disharmonic).

6. Duplex and multiplex limbs arise frequently from the trans-
planted buds. They are of all grades and kinds and occur in
different proportions in the several experiments. In the hetero-
topic grafts they are more frequent in the harmonic combinations,
while in the orthotopic position they are much more frequent in
the disharmonic combinations.

7. In nearly all cases one member of a pair or group can be
distinguished as the original (primary) and the other one or ones

as buds. The reduplicating bud is in each case the mirror image of the original, and, when the reduplicating bud is itself doubled, then the one next to the original is the mirror image of the latter, while the one further away is mirrored, with respect to its mate, approximately in accordance with Bateson's rule.

8. Limbs placed in abnormal location, where the specific blood and nerve supply is lacking, are frequently resorbed, and when they do develop, are usually stiff and functionless, or at best show imperfect function. The shoulder-girdle in such limbs is reduced in size and the more outlying elements are lacking.

9. Limb buds placed in normal location (orthotopic) are rarely resorbed and nearly always become functional.

10. Limb buds from the same side of the body normally oriented in orthotopic position develop normally with but slight retardation.

11. When the limb bud from the same side is rotated 180° in its normal location, the results vary considerably, and in the majority of cases reduplications occur. The single limbs are of two kinds, reversed and normal. The former develop in accordance with rule 2 (p. 4), but only one case of this kind has been observed, the others that conform to the rules being reduplicated (rule 3). In the other cases the normal position was reached by the rotation of the limb as a whole about the shoulder-joint. These cases are exceptions to the rules.

12. Of the twenty-seven cases of reduplications in the above group, the original bud grew anteriorly and was reversed. In the three remaining cases the primary limb righted itself by rotation and the reduplicating member was reversed.

13. Regulation by rotation usually takes place when at the operation the limb bud has been rotated anteriorly over the dorsal semicircumference not quite 180°.

14. Limb buds from the opposite side of the body, with the dorsoventral axis normally oriented, produced but one unreversed single limb, in accordance with rule 1 (p. 4), the rest being reduplications (rule 3). In one-sixth of the latter the original bud, which was disharmonic, was resorbed and remained as a small nodule or spur on the reduplicating appendage. The dupli-

cate bud in these cases, having its asymmetry reversed and oc-
cupying the right position, became a normally functioning fore
limb, perfectly adjusted both functionally and structurally to
its organic environment.

15. Limb buds, taken from the opposite side of the body and
implanted with the dorsoventral axis inverted, so as to leave the
anteroposterior axis in normal relation, formed, with the excep-
tion of one reduplication, single limbs, all of which were reversed
These limbs were often considerably retarded in development,
but, as regards both function and form, they became perfectly
adjusted to their new surroundings (rule 2).

16. In the superposed grafts two limb buds are fused into one.
In the two harmonic combinations normal single limbs arise.
Though at first usually above normal in size, they soon become
regulated in this respect. In the disharmonic combinations du-
plex appendages were formed in a large majority of cases. One
case of adjustment by rotation and one case of regulation by
reduction of one member of a pair were found.

17. In experiments with half buds there are sixteen combina-
tions possible with the restrictions imposed by the character of
the experiment. In addition to the two pairs of attributes of
operation common to all of the experiments (*hom.* or *het.*, *dd* or
dv) there are three others: the bud may be halved vertically or
horizontally; the anterior or the dorsal half, or the posterior or
the ventral half may be transplanted, the other remaining intact;
two like halves or two unlike halves may be united. An analysis
of the results shows that no one of these qualities in itself deter-
mines the result, but that it is the harmonic or disharmonic
character of the combination that determines whether normal or
reduplicated appendages arise. Thus, allowing for differences
in the number of experiments in each class, 93.4 per cent of the
harmonic combinations produced normal limbs, while in the
disharmonic groups about that same proportion produced redu-
plications, of which, however, a considerable number were regu-
lated secondarily through resorption of the disharmonic member.

18. That the limb bud is an equipotential system is shown by
the fact that a normal limb may develop after the following oper-

ations, provided the combination is harmonic: 1) extirpation of
any half of the bud; 2) fusion of two whole buds; 3) combination
of two like halves, the other half being entirely missing; 4) inver-
sion of the limb bud; 5) inoculation of mesoderm cells from the
limb under the skin in some other region of the embryo.

19. Except for the circumstance that the dorsoventral differen-
tiation of the limb bud is a function of the orientation of the bud
with respect to its organic environment, the limb bud is a highly
specific self-differentiating system. Its definitive form must,
therefore, be represented in the organic elements (intimate struc-
ture) of the limb rudiment.

20. One quality of these elements is their polarization, as
shown by the definite relation to the direction of out-growth,
assumed by the anteroposterior axis of the limb bud. It is sug-
gested that the asymmetry of the limb rudiment and of other
similar systems may be gradually brought about by the change
in constitution of the structural elements in a manner similar to
the building up of asymmetric molecules in organic compounds.

21. Reduplications are produced as a result of that funda-
mental attribute of living matter, the power to divide (Bateson).
They are induced, in the case of the limb bud especially, by a
disharmonic relation between graft and host.

22. There is no fundamental distinction between double super-
numerary limbs constituting a symmetrical pair and the single
supernumerary symmetrical with the normal one with which it
is associated. Bateson's rules may be stated in simplified form
in accordance with this conclusion (p. 97).

23. Exceptions to Bateson's rule regarding symmetry rela-
tions of supernumerary parts are very rare. Those found in the
present study, where two limbs of the same side occurred in linear
series, are probably due to the appendages having been far
enough apart not to influence one another in development, and
at the same time having been under the influence of the same
organic environment.

24. Review of the data on regeneration of supernumerary
appendages shows that the reversal of asymmetry in one of the
members of an enantiomorphic pair is not dependent upon the

reversal of direction of growth, regeneration, or differentiation. The reversed member may grow and differentiate in the same direction as the original, another axis than that on which growth is taking place being the one that is reversed. Reversal may thus occur without axial heteromorphosis and vice versa.

25. In any system, like that of the limb bud at the time of transplantation, in which at least one axis is left undifferentiated, rotation of the elements of which the system is made up might account for reversal. The rotation of cells observed by Hadži and Przibram is, however, concerned primarily with wound healing, and heret is no evidence that it is correlated with the occurrence of axial heteromorphosis or reversal of asymmetry.

26. As an alternative to the hypothesis of rotation, we might consider reversal as due to reversal of molecular asymmetry according to analogy with the behavior of optically active compounds.

27. There is an analogy between the production of enantiomorphic limbs and the production of situs inversus viscerum, as effected by Spemann. Either the reversal may be due to reversal of the intimate structure, or it may take place in spite of the intimate structure through the direct action of mechanical factors on the individual parts of the differentiating system.

28. The transplanted limbs show both regulation of form and functional adaptation. The two often go hand in hand, but not necessarily, for some cases show regulation of form without function, and others functional regulation without form regulation.

29. Functional regulation is largely a matter of innervation, and it occurs only in orthotopic grafts or in those approximately in that position (Detwiler).

30. Form regulation is either primary, as in the case of harmonic combinations, or secondary, as in the disharmonic. In the latter it takes place in one of two ways, either by rotation of the developing limb or by means of reduplication and reduction of the disharmonic member.

31. Form regulation by rotation has been observed to occur only in orthotopic grafts; reduplications in disharmonic combinations are more frequent in orthotopic than in heterotopic

transplantations. While these circumstances lead to an harmonic end-result more frequently where there is functional adaptation as well, this cannot be used as a cogent argument for a teleological theory of development.

LIST OF REFERENCES

BARFURTH, DIETRICH 1894 Die experimentelle Regeneration überschüssiger Gliedmassenteile (Polydaktylie) bei den Amphibien. Arch. f. Entw.-Mech., Bd. 1.

BATESON, WILLIAM 1894 Materials for the study of variation. London.
1913 Problems of genetics. New Haven.

BENDER, O. 1906 Zur Kenntnis der Hypermelie beim Frosch. Morpholog. Jahrbuch, B. 35.

BRAUS, H. 1904 Einige Ergebnisse der Transplantation von Organanlagen bei Bombinatorlarven. Verh. d. Anatom. Ges. 18. Versammlung i. Jena.
1905 Experimentelle Beiträge zur Frage nach der Entwicklung peripherer Nerven. Anatom. Anz., Bd., 26.
1906 Vordere Extremität und Operculum bei Bombinatorlarven. Morpholog. Jahrbuch, Bd. 35.
1909 Gliedmassenpfropfung und Grundfragen der Skeletbildung. I. Die Skeletanlage vor Auftreten des Vorknorpels und ihre Beziehung zu den späteren Differenzierungen. Morpholog. Jahrbuch, Bd. 39.

CHILD, C. M. 1908 Driesch's harmonic equipotential systems in form-regulation. Biol. Zentralbl., Bd. 28.
1911 Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. The axial gradient in Planaria dorotocephala as a limiting factor in regulation. Jour. Exp. Zoöl., vol. 10.
1913 Studies, etc. VI. The nature of the axial gradients in Planaria and their relation to anteroposterior dominance, polarity and symmetry. Arch. f. Entw. Mech., Bd. 37.
1915 Individuality in organisms. Chicago.

COLE, L. J. 1910 Description of an abnormal lobster cheliped. Biol. Bull., vol. 18.

CONKLIN, E. G. 1916 Effects of centrifugal force on the polarity of the egg of Crepidula. Proc. Nat. Acad. Sci., vol. 2.
1917 Effects of centrifugal force on the structure and development of the eggs of Crepidula. Jour. Exp. Zoöl., vol. 22.

DAWSON, A. B. 1920 An exception to Bateson's rule of secondary symmetry. Biol. Bull., vol. 38.

DELLA VALLE, P. 1913 La doppia rigenerazione inversa nelle fratture delle zampe di Triton. Bollettino della Società di Naturalisti in Napoli, vol. 25 (1911-12).

DETWILER, S. R. 1918 Experiments on the development of the shoulder girdle and the anterior limb of Amblystoma punctatum. Jour. Exp. Zoöl., vol. 25.

DETWILER, S. R.   1919   The effect of transplanting limbs upon the formation of nerve plexuses and the development of peripheral neurones.   Proc. Nat. Acad. Sci., vol. 5.
    1920   Experiments on the transplantation of limbs in Amblystoma. The formation of nerve plexuses and the function of the limbs.   Jour. Exp. Zoöl., vol. 31.

DRIESCH, HANS   1899   Die Lokalisation morphogenetischer Vorgänge.   Ein Beweis vitalistischen Geschehens.   Arch. f. Entw.-Mech., Bd. 8.
    1901   Die organischen Regulationen.   Leipzig.
    1902   Über ein neues harmonisch-äquipotentielles System und über solche Systeme überhaupt.   Arch. f. Entw.-Mech., Bd. 14.
    1905   Die Entwickelungsphysiologie von 1902–1905.   Ergeb. d. Anat. u. Entw., Bd. 14.
    1906   Studien zur Entwicklungsphysiologie der Bilateralität.   Arch. f. Entw.-Mech., Bd. 21.
    1908 a   Zur Theorie der organischen Symmetrie.   Arch. f. Entw.-Mech., Bd. 26.
    1908 b   The science and philosophy of the organism.   London.

EMMEL, V. E.   1907   Regenerated and abnormal appendages in the lobster. Special paper no. 31.   Reprinted from the Thirty-seventh Annual Report of the Commissioner of Inland Fisheries of Rhode Island.

FRITSCH, C.   1911   Experimentelle Studien über Regenerationsvorgänge des Gliedmassenskelets der Amphibien.   Zool. Jahrb., Bd. 30.

GIARD, M. A.   1895   Polydactylie provoquée chez Pleurodeles Walthii Michahelles.   Comptes Rendus de la Société de Biol., T. 10, Sér. 10.

HARRISON, R. G.   1907   Experiments in transplanting limbs and their bearing upon the problems of the development of nerves.   Jour. Exp. Zoöl., vol. 4.
    1915   Experiments on the development of the limbs in Amphibia. Proc. Nat. Acad. Sci., vol. 1.
    1916   On the reversal of laterality in the limbs of Amblystoma embryos. Anat. Rec., vol. 10.
    1917 a   Further experiments on the laterality of transplanted limbs. Anat. Rec., vol. 11.
    1917 b   Transplantation of limbs.   Proc. Nat. Acad. Sci., vol. 3.
    1918   Experiments on the development of the fore limb of Amblystoma, a self-differentiating equipotential system.   Jour. Exp. Zoöl., vol. 25.

KURZ, OSKAR   1912   Die beinbildenden Potenzen entwickelter Tritonen.   Arch. f. Entw.-Mech., Bd. 34.

LILLIE, FRANK R.   1906   Observations and experiments concerning the elementary phenomena of embryonic development in Chaetopterus.   Jour. Exp. Zoöl., vol. 3.
    1909   Polarity and bilaterality of the annelid egg.   Experiments with centrifugal force.   Biol. Bull., vol. 16.

LISSITZKY, EUGEN   1910   Durch experimentelle Eingriffe hervorgerufene überzählige Extremitäten bei Amphibien.   Arch. f. mikr. Anat. u. Entw., Bd. 75.

MEGUŠAR, FRANZ   1907   Die Regeneration der Coleopteren.   Arch. f. Entw.-Mech., Bd. 25.

MORGAN, T. H.   1905   'Polarity' considered as a phenomenon of gradation of
materials.   Jour. Exp. Zoöl., vol. 2.
1908 a   Experiments in grafting.   The American Naturalist, vol. 42.
1908 b   The location of embryo-forming regions in the egg.   Science,
N. S., vol. 28.
1910   Cytological studies of centrifuged eggs.   Jour. Exp. Zoöl., vol. 9.
MORGAN, T. H., AND SPOONER, G. B.   1909   The polarity of the centrifuged egg.
Arch. f. Entw.-Mech., Bd. 28.
MORRILL, C. V.   1919   Symmetry reversal and mirror imaging in monstrous
trout and a comparison with similar conditions in human double mon-
sters.   Anat. Rec., vol. 16.
NEWMAN, H. H.   1916   Heredity and organic symmetry in armadillo quadrup-
lets.   II. Mode of inheritance of double scutes and a discussion of
organic symmetry.   Biol. Bull., vol. 30.
PRESSLER, KURT   1911   Beobachtungen und Versuche über den normalen und
inversen Situs viscerum et cordis bei Anurenlarven.   Arch. f. Entw.-
Mech., Bd. 32.
PRZIBRAM, HANS   1902   Experimentelle Studien über Regeneration.   Arch. f.
Entw.-Mech., Bd. 13.
1906   Regeneration als allgemeine Erscheinung in den drei Reichen der
Natur.   Vortrag. Versamm. Deutscher Naturf. u. Aerzte. Stuttgart.
Naturwiss. Rundschau, Bd. 21.
1909   Experimental-Zoologie, 2.   Renegeration.
1910 a   Die Verteilung organbildender Fähigkeiten auf Körperre
gionen.   Verh. der k. k. zoologisch-botanischen Ges. in Wien.
1910 b   Die Verteilung formbildender Fähigkeiten am Tierkörper in
dorso-ventraler Richtung.   Arch. f. Entw.-Mech., Bd. 30.
1911   Experiments on asymmetrical forms as affording a clue to the
problem of bilaterality.   Jour. Exp. Zoöl., vol. 10.
1913   Experimental-Zoologie, 4.   Vitalität, Kap. 3, Polarität.
REED, MARGARET A.   1904   The regeneration of the first leg of the crayfish.
Arch. f. Entw.-Mech., Bd. 18.
SPEMANN, H.   1910   Die Entwicklung des invertierten Hörgrübchens zum Laby-
rinth.   Arch. f. Entw.-Mech., Bd. 30.
SPEMANN, HANS, UND FALKENBURG, HERMANN   1919   Über asymmetrische Ent-
wicklung und Situs inversus viscerum bei Zwillingen und Doppel-
bildungen.   Arch. f. Entw.-Mech., Bd. 45.
STREETER, G. L.   1907   Some factors in the development of the amphibian ear
vesicle and further experiments on equilibration.   Jour. Exp. Zoöl.,
vol. 4.
1914   Experimental evidence concerning the determination of posture
of the membranous labyrinth in amphibian embryos.   Jour. Exp.
Zoöl., vol. 16.
TANNREUTHER, GEORGE W.   1919   Partial and complete duplicity in chick
embryos.   Anat. Rec., vol. 16.
TORNIER, GUSTAV   1897   Über experimentell erzeugte dreischwänzige Eidech-
sen und Doppelgliedmassen von Molchen.   Zool. Anz., Bd. 20.
1900   Das Entstehen von Käfermissbildungen besonders Hyperanten-
nie und Hypermelie.   Arch. f. Entw.-Mech., Bd. 9.

TORNIER, GUSTAV 1905 An Knoblauchskröten experimentell entstandene überzählige Hintergliedmassen. Arch. f. Entw.-Mech., Bd. 20.
1906 Experimentelles und kritisches über tierische Regeneration. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin.

WILDER, HARRIS HAWTHORNE 1904 Duplicate twins and double monsters. Am. Jour. Anat., vol. 3.

ZELENY, CHARLES 1905 The regeneration of a double chela in the fiddler crab (Gelasimus pugilator) in place of a normal single one. Biol. Bull., vol. 9.

APPENDIX

HISTORIES OF SELECTED INDIVIDUAL CASES

A. *Heterotopic transplantations*

1. Homopleural, dorsodorsal

Experiment Tr. E. 148. May 4, 1915. Right limb bud to right flank; orientation normal.

May 5. Healed except for two small oval areas.

May 8. Transplanted bud points caudally like normal limb.

May 12. Sketch (fig. 5).

May 15. Transplanted limb slightly bidigitate—not so long or as far differentiated as normal.

May 21. Transplanted limb has grown considerably, normal posture; two digits well marked, third appearing.

May 26. Third and fourth digits show more plainly than in sketch made on May 24 (fig. 6).

June 1. Limb a normal right (homopleural) limb in normal posture (fig. 7).

Experiment Tr. E. 182. April 13, 1916. Right limb bud to right flank; orientation normal.

April 14. Perfectly healed.

April 16. Tranplanted bud as large as normal, points caudally.

April 25. Not so large as normal, points dorsocaudally.

May 1. Much shorter than normal. Points caudally, arising from a ridge-like prominence on side of body.

May 7. Two distinct digits and trace of third. A new bud is growing from base anterolaterally.

May 15. Original limb (the posterior one in fig. 9) has three distinct digits and beginning of fourth, shows elbow bend. It is a normal right (homopleural) in nearly normal posture, though it sticks out stiffly to side. The two limbs are entirely separate from their origin. The anterior member, which is the reduplicating one, shows only a faint trace of the third digit on the anterior border.

Experiment Tr. E. 210. May 10, 1916. Right limb bud to right flank; orientation normal.

May 11. Healed except for small area of uncovered yolk at caudal border of wound.

May 15. Transplanted bud somewhat smaller than normal, points in same direction.

May 18. Posture as of normal right limb. At base a rounded prominence which points headward.

May 22. The prominence has become a reduplicating limb, showing first trace of digits.

May 28. Both limbs well developed. Original is longer and is a right (homopleural) with three digits and faint trace of fourth. Reduplicating member branches from other at elbow and is a mirror image of latter, except that it is less advanced in development.

2. Homopleural, dorsoventral

Experiment Tr. E. 219. May 17, 1916. Right limb bud to right flank, inverted.

May 18. Healing fair; a considerable area anterior to grafted bud not covered with ectoderm.

May 21. Bud looks nearly normal, but points dorso-anteriorly. See sketches made on May 22 (figs. 10 and 11).

May 25. Transplanted bud growing as rapidly as normal. The two point toward each other (figs. 12 and 13).

May 29. Transplanted limb growing well; two distinct digits; points dorso-anteriorly and is apparently a mirror image of normal limb on same side (figs. 14 and 15).

June 5. Rapid growth has continued; limb reaches gills; third digit well marked, fourth beginning to show, elbow bend is slight; hand in an almost vertical plane, palm being anteromedial. Limb is an undoubted left, i.e., reversed (figs. 16 and 17).

Experiment Tr. E. 139. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing perfect, transplanted tissue rather prominent.

May 5. Limb bud points slightly dorso-anteriorly.

May 8. Points distinctly dorso-anteriorly.

May 17. Limb points laterally at angle of a little less than 60° to axis of body. Third digit developing.

May 26. Four digits present (fig. 18). Limb does not look atrophic, but gives no evidence of motility or sensitivity. Position as when last observed. Specimen preserved.

Examination of serial sections shows that the anteromedial surface of the hand is the palm. There can be no doubt, consequently, that the limb is a normal left, having been reversed.

Experiment Tr. E. 140. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing of wound good; slight areas still uncovered by ectoderm at ventral border of wound.

May 5. Transplanted tissue growing; but points anterodorsally.

May 11. Digitations plain; limb points as before.

May 17. Limb now points almost transversely, with ulnar digits on anterior border.

May 26. Perfect limb with extensor surface of elbow and ulnar digits dorso-anterior. No motility (fig. 19). Sections of preserved specimen show that ventral surface is palm, and that there can be no question about its being a left limb, i.e., reversed.

Experiment Tr. E. 220. May 17, 1916. Right limb bud to right flank inverted.

May 18. Wound perfectly healed.

May 21. Transplanted bud very prominent. Sticks out to side and slightly anteriorly.

May 25. Transplanted limb points dorso-anteriorly and not much to side. A small reduplicating bud points ventroanteriorly from near base.

May 29. Limb points as before. Two well-marked digits which are slightly irregular. Probably no reduplication.

June 5. Limb sticks out to side almost horizontally; first two digits short, third distinct, fourth barely indicated. Reduplicating hand attached to radial border of the same limb and pointing ventrally has two digits.

N. B. It is uncertain whether this member developed out of the bud noted on May 25.

3. Heteropleural, dorsodorsal

Experiment Tr. E. 227. May 19, 1916. Right limb bud to left side, dorsodorsal.

May 20. Well healed.

May 23. Transplanted bud points dorso-anteriorly and is more massive than normal.

May 26. Grafted limb about same length as normal; points anteriorly and laterally, scarcely dorsally (figs. 20 and 21 drawn one day later).

June 2. Grafted limb now reaches to the axilla of the normal limb; trace of third digit (fig. 22, made day before).

June 5. Limb a normal right (is not reversed), pointing antero-laterally, crossing the normal limb on the medial surface (fig. 23).

Experiment Tr. E. 107. April 9, 1913. Right fore limb to left side, dorsodorsal orientation. Diameter of transplanted bud 3 somites (3 to 5).

April 10. Crescentic uncovered area of yolk ventral to bud, which otherwise is well healed in.

April 14. Grafted bud shows indications of pointing headward.

April 21. Limb growing, pointing anteriorly exactly in reversed position. No digitations yet.

April 28. Limb is clearly a right; third digit now plain, with slight nodule to indicate fourth.

May 5. Possibly slight motility of grafted limb. It is now seen to be a perfect right, sticking out sharply to side, upper arm is inclined dorso-anteriorly, fore arm slightly posteriorly; palmar surface of hand ventro-anterior (figs. 24 and 25).

Experiment Tr. E. 127. May 12, 1914. Right limb bud to left side, dorsodorsal orientation; diameter of bud 3 somites.

May 13. Healing perfect.

May 16. Transplanted bud growing well with slight indication of pointing anteriorly.

May 18. Limb points distinctly anteriorly.

May 22. Two digits show; a reduplicating bud is growing out from base of graft.

June 1. The primary limb now shows fourth digit and is a perfect right (fig. 26, *PR*). The reduplicating member (*DU*) is a left, though not quite so far along in its development. The primary limb now points laterally and posteriorly with its ulnar border headward.

4. Heteropleural, dorsoventral

Experimental Tr. E. 193. April 14, 1916. Right limb to left flank, dorsoventral orientation; diameter of transplanted bud $3\frac{1}{4}$ somites.

April 15. Wound perfectly healed.

April 17. Transplanted bud, rather larger than normal, points posteriorly.

April 21. Transplanted bud has large base, but free portion is slender; points distinctly posteriorly.

April 28. Transplanted bud growing, though much smaller than normal. Points dorsoposteriorly and has two digits.

May 1. Digits more distinct. Slight bending at elbow. Has position of normal left limb.

May 7. Three digits and trace of fourth. A normal left limb in almost normal posture (fig. 27).

Experiment Tr. 167. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Wound completely healed. Transplanted bud prominent.

Feb. 28. Transplanted bud a large blunt prominence which points posteriorly.

March 3. Transplanted bud is very small; may be resorbed.

March 7. Grafted limb much smaller than normal, but growing.

March 11. Considerable growth, but still smaller than normal. Slight indication of elbow bend and digits.

March 15. Digits marked. Limb looks like a normal left reversed, though it sticks out to side more than the normal limb.

March 24. Third digit now plain. Arm points to side. Hand transverse and vertical with respect to body.

March 29. Preserved. Transplanted limb a normal left in nearly normal posture. Fourth digit indicated. Upper arm still sticks out more to side than normal, but otherwise little difference except in size.

Experiment Tr. E. 129. May 13, 1914. Right limb to left side, dorsoventral orientation. Transplanted disc 3 somites in diameter.

May 14. Wound perfectly healed.

May 18. Transplanted bud has grown considerably, points posteriorly.

May 25. Grafted limb points laterally and about 30° caudally, is bidigitate. Hand and perhaps forearm are being reduplicated on anterolateral border.

June 6. Specimen preserved. Imperfectly symmetried double hand, mirrored from radial plane. Arm stretches out to side with elbow bending ventrally. The posterior (original) hand is a left (reversed), as is the arm as a whole; it has four digits normally placed; the anterior (reduplicating) hand is less perfect with only three digits, one long one in the middle and a short one on each side, the first digit being imperfect.

Experiment Tr. E. 163. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Well healed. Very small uncovered area ventral to bud.

February 28. Transplanted bud larger than normal and a little further ventral.

March 3. Bud is growing ventroposteriorly.

March 7. Bud long and slender, growing posteriorly almost on ventral surface of body; probably defective.

March 11. Same.

March 15. Original limb has not changed essentially. A second outgrowth of considerable length arises some distance dorsal to former and projects laterally. . Judging by its size, it must have been present and overlooked at last observation.

March 20. Original limb a long bent appendage tapering gradually to a point. New bud has made considerable growth and is bidigitate, the digits spreading more than normally. Indication of elbow bend.

March 28. Secondary appendage has developed a third digit, which, with elbow bend, shows that it is probably a left (reversed).

April 4. Specimen preserved (figs. 33 and 34). Transverse sections show that limb is undoubtedly a left, since it is the dorsoposterior surface of the hand that is the palm—a very unusual posture. The two limbs have separate girdles which articulate with one another, but are not fused.

Experiment Tr. E. 217. May 17, 1916. Right limb bud to left flank, dorsoventral.

May 18. Perfectly healed.

May 21. Grafted bud almost exactly like normal, points dorsoposteriorly.

May 29. Limb short, sticks out more sharply to side. Digitations will probably be abnormal.

May 31. Possibly symmetrical reduplication of digits (fig. 31).

June 5. Uncertainty about reduplication. One distinct digit (third) on posterior (ulnar) border and also a slight nodule (fourth digit). Prominence on opposite border may not be digits (fig. 32).

June 8. One distinct digit and trace of another formed out of hump on radial side so that hand is now practically symmetrical.

. Limb amputated; afterward regenerated; form much like original.

B. *Orthotopic transplantations*

7. Homopleural, dorsodorsal

Experiment N. E. 3. May 19, 1918. Right limb bud to right side —normal orientation. Pronephros intact.

May 21. Perfectly healed; limb bud normal.

May 25. Both limbs same except that transplanted one is slightly shorter.

May 28. No difference between the two limbs.

June 7. Same. Specimen preserved.

8. Homopleural, dorsoventral

Experiment I. E. 64. May 13, 1916. Left limb bud to left side, inverted. Pronephros removed.

May 15. Small uncovered area ventral to bud, which is larger than normal and slightly posterior to it.

May 18. Limb bud points dorso-anteriorly (fig. 35).

May 24. Transplanted limb points anteriorly into gills. Slight trace of digits; no reduplicating bud (fig. 36, drawn one day earlier).

May 28. Limb points anterolaterally below gills.

May 29. Limb is undoubtedly a right (reversed) and is perfectly normal except as to posture. Ulnar digits appearing on the antero-dorsal border of hand (figs. 37 and 38).

June 1. Transplanted limb sticks out more to side than before and can be brought back further toward normal position. Motility was first observed two days ago.

June 4. Larva has grown rapidly. Transplanted limb functions well; elbow bends toward tail instead of head. Limb a perfect right (figs. 39 to 41, drawn one day later).

Experiment I. E. 60. May 11, 1916. Left limb to left side, inverted. Pronephros removed.

May 12. Well healed, normal-looking bud.

May 15. Grafted bud points dorso-anteriorly.

May 16. Sketches (figs. 42 and 43).

May 18. Beginning of bud like outgrowth (reduplication) posteriorly (fig. 44, *DU*).

May 24. Main limb (*PR*) points more sharply to side and rather more dorsally than normal. Posterior (reduplicating) bud (*DU*) has grown considerably (figs. 45 and 46, drawn May 23).

May 29. Specimen preserved. Limb double from lower part of fore arm. The primary member, which is anterolateral, is a right (reversed), while the other, produced from the posterior bud, is a left (reversed back). Former has short first digit and well-marked third. On the reduplicating hand a trace of third digit present (figs. 47 and 48). Mirror plane is radiodorsal.

Frontal sections show the radii of the two arms are fused at the proximal end, the two ulnae being entirely separate. The humerus is single, i.e., fused throughout and is much more massive than normal. Glenoid cavity is also much larger, but normally oriented. Barring slight abnormalities, the shoulder-girdle seems to be a normal left (not reversed).

Experiment I. E. 63. May 12, 1916. Left limb to left side, inverted. Pronephros removed.

May 13. Fairly well healed.

May 15. Transplanted bud smaller than normal; points dorsally, sharply laterally, and slightly anteriorly (figs. 49 and 50, drawn May 16).

May 19. Points dorso-anteriorly. Slight indication of posterior reduplicating bud at base (figs. 51 and 52).

May 24.   Main limb points much more dorsally and laterally than normal (figs. 53 and 54, drawn May 22); no digits.   Two reduplicating buds; posterior one (*P.DU*) points as in I. E. 60 (figs. 44 and 45); anterior one (*A.DU*) smaller and spur-like.

May 29.   Specimen preserved (fig. 55).   Main limb is a right (reversed) with two long digits and third digit on anterodorsal (ulnar) border.   The posterior reduplicating member with three digits is free and is in approximately normal position for left limb (reversed back). It is mirrored in a radiodorsal plane.   The anterior hand is bidigitate, and is mirrored in an ulnopalmar plane.   Arm and fore arm of complex is short and thick.

Experiment I. E. 49.   May 14, 1915.   Left limb bud to left side of same individual turned 180°.   Pronephros removed.

May 15.   Fairly well healed; irregular band of uncovered yolk at posterior border of wound.

May 21.   Limb bud developing well, points dorsally with very slight anterior inclination.

May 25.   Limb points dorsally (fig. 56); two digitations—hand flattened in vertical plane, making angle of 45° with body axis.

May 29.   Limb points dorsoposteriorly, as if approaching normal position.

May 30.   Limb has rotated still further toward normal position.

June 3.   Limb in nearly normal position (fig. 57, drawn June 4).

June 11.   Limb tends to lie partially adducted with dorsal surface of manus on bottom.

June 17.   Limb now perfectly normal in every respect—form, size, posture, motility (fig. 58, drawn June 21, and fig. 59, drawn from specimen preserved thirty-nine days after operation).

Experiment I. E. 55.   May 23, 1915.   Left limb bud removed and replaced inverted. Pronephros removed.

May 24.   Very well healed.   Only a very narrow uncovered strip at posterior border.

May 27.   Limb bud smaller and more pointed than normal.

May 30.   Limb bud increasing, but not so large as normal.   Sticks out more to side.

June 2.   Limb bud points dorsoposteriorly, ventral part of bud prominent.

June 7.   Limb has grown considerably, points more posteriorly than before.   Two digits; manus flattened in transverse plane.

June 11.   Posture of limb still abnormal.   It points dorsopostero-laterally at about 45° to median and horizontal planes.   Trace of third digit.

June 17.   Limb normal in every respect—form, size, posture, motility.

Experiment I. E. 85.   April 10, 1917.   Left limb bud to left side, rotated 180° + (fig. 60).   Pronephros removed; pronephros grafted along with limb bud.

April 11.   Well healed.

April 14.   Grafted bud about as distinct as normal, points dorsally and slightly anteriorly.

April 16. Bud points dorso-anteriorly ca. 60° to horizontal; attachment slightly posterior to normal. Distinct pronephric swelling ventral to limb.

April 19. Original bud points as before. Posterior reduplicating bud in approximately normal position. Anteriorly, at base of original bud, is a third bud pointing anterolaterally.

April 24. Middle (primary) member points dorsally and has two long digits; anterior member is attached along anterior border of middle; posterior member, in normal posture shows beginning of digits.

May 7. Specimen preserved (fig. 61). Posterior member, an essentially normal left limb from its origin above elbow down, has four digits; it is mirrored from the middle member in a radiodorsal plane. Anterior and middle limbs fused except for distal portion of manus; mirror plane, palmar. The middle member has two long digits and nodules on ulnar border, and is probably a right (reversed). Anterior member has three long digits and ulnar nodule, the third being partly fused with the second of the middle hand. Anterior hand possibly hyperdactylous.

Experiment I. E. 86. April 10, 1917. Left limb bud to left side, rotated 180°— (fig. 60). Pronephros removed; pronephros transplanted with limb tissue.

April 11. Well healed.

April 14. Limb bud prominent, placed a little further posteriorly than normal; points slightly dorsally.

April 16. Transplanted bud points dorsoposteriorly, but more dorsally and slightly more laterally than normal. Pronephric swelling ventroanterior to bud.

April 19. Limb points more dorsally than normal. Small nodule at base is part of pronephros.

April 24. Limb in normal posture not quite so long as normal. Third digit beginning. Reduplicating digit comes off palmar side between first and second digits.

April 29. Second and third digits reduplicated (palmar); limb otherwise very nearly normal. Motility apparently not so good as normal.

May 5. Limb clearly a left (not reversed); arm a little shorter and thicker. Motility now better.

May 7. Specimen preserved (fig. 62).

9. Heteropleural, dorsodorsal

Experiment R. E. 87. May 19, 1915. Right limb to left side, dorsodorsal orientation. Pronephros left intact.

May 21. Small round uncovered area posterior to bud.

May 25. Transplanted bud points anteriorly and laterally (ca. 45°), and is nearly as large as normal (fig. 63, drawn May 26).

May 28. Limb points as before and is growing into gills, one of which is caught in notch between limb and neck and is bent ventrally. Limb itself bent slightly at tip. Digitations faintly indicated.

June 2. Limb thinner than normal and somewhat limp. Bends backward at tip. Two digits only.

June 4. Specimen preserved (fig. 64). The arm sticks out horizontally to side; elbow bends posteriorly; two digits in same horizontal plane with very faint indication of ulnar digits. Limb clearly a right (not reversed), though it is distinctly smaller than normal and is otherwise defective.

Experiment R. E. 70. May 12, 1915. Operation: right limb bud to left side, dorsodorsal orientation. Pronephros left in.

May 13. Still a crescentic area of uncovered yolk at ventral border of bud.

May 15. Transplanted bud more prominent than normal; indications of pointing anteriorly (fig. 67, drawn May 17, at which time the posterior bud could just be made out).

May 21. Points anterolaterally. Attached to base is an almost equally large reduplicating bud, pointing posteriorly and normally located (fig. 68, drawn May 22).

May 27. Both buds growing. The original (anterior) one is still a little further advanced than the other. Figure 69, drawn May 28, shows beginning of digits.

June 7. The original limb is still the larger one, the reduplicated one has two digits and trace of third. The complex has some motility.

June 11. Specimen preserved (fig. 70). The arm is double from the elbow down; the posterior or reduplicating member is not so well developed as the other. The anterior member is a right (not reversed) while the other is mirrored in a radiodorsal plane and occupies a position approximately normal for the left limb.

Experiment R. E. 133. May 25, 1916. Right limb bud to left side dorsodorsal orientation. Pronephros removed.

May 26. Fairly well healed. Small uncovered area ventral to bud.

May 29. Transplanted bud well developed, points dorsoanteriorly about 45°. It is in proper position and about same size as normal.

June 2. Limb points dorso-anteriorly, but more sharply dorsally, extending into notch behind last gill. Two digits show distinctly as in normal limb. Reduplicating bud from posterior border of base has position of normal left.

June 5. Original limb (fig. 75, *PR*) points dorsolaterally toward gills and looks nearly normal. Posterior (reduplicating) bud (*P.DU*) is rather short and arises further tailward than normal limb; digitations beginning. A third small limb (*A.DU*) anterior to the original, projects dorsolaterally, parallel to it.

June 7. Original limb a normal right (not reversed) having two long digits with trace of third and fourth. The posterior bud has grown considerably and is in normal position. Anterior bud shows traces of digits.

June 12. All three limbs have grown and differentiated. Posterior has nodule for third digit and is a normal left (reversed). It is mirrored in a radiodorsal plane while the anterior, limb, which is more

nearly parallel to the original, is mirrored in an ulnopalmar plane (fig. 76, drawn June 13).

Experiment R. E. 134. May 25, 1916. Right limb bud to left side, dorsodorsal. Pronephros removed.

May 26. Perfectly healed.

May 29. Transplanted bud, normal size and position, points antero-dorsally ca. 45°.

June 2. Limb points dorsally and somewhat laterally. Reduplicating bud on anterior border near base also points dorsally and slightly anteriorly.

June 5. Limb points anteriorly into gills, is short and massive. Three distinct digits, irregular.

June 12. Specimen preserved (fig. 77). Arm short and thick, internal reduplication, certainly from elbow down, mirrored in ulno-palmar plane. The two hands nearly separate; each has two long digits and nodular third. The main limb is a right (not reversed) and the other a left. No posterior reduplication in this case.

Experiment R. E. 69. May 12, 1915. Right limb to left side, dorso-dorsal orientation. Pronephros left in.

May 13. Wound well healed. Only a small area at ventroposterior border of wound uncovered.

May 15. Transplanted and normal budsa eqully prominent; former points anteriorly.

May 21. Transplanted limb double. Smaller (originally main) part (fig. 86, *PR*) points anteriorly and is borne upon the posterior portion, *DU'*, which is an almost normal looking limb, though smaller than the normal on the opposite side.

May 24. The posterior bud has grown materially and is normal looking with two digits. The anterior bud is relatively much less developed (fig. 87).

May 27. The original bud is reduced to a small spur (*PR*) on the lateral surface of the reduplicating bud, which is almost as large as the normal limb on the opposite side (fig. 88, drawn one day later).

June 3. Transplanted limb perfectly normal. Spur much reduced.

June 7. Spur reduced to a nodule (fig. 89, *PR*).

10. Heteropleural, dorsoventral

Experiment R. E. 80. May 18, 1915. Right limb bud to left side, dorsoventral orientation. Pronephros removed.

May 19. Crescentic band ventroposterior to bud still uncovered.

May 21. Limb bud almost normal in size points posteriorly.

May 24. Limb bud points posteriorly, but more sharply laterally than normal (fig. 93, *TR*).

May 30. Limb points dorsoposteriorly angle of 45°.

June 2. Limb stiff and projects rather more to side than normal; has two digits and slight indication of third on dorsal border (fig. 94, drawn June 1).

June 11. Limb mobile, in normal posture. Brachium shorter than normal. Third digit distinct, fourth indicated.

June 21. Four digits all plain. Upper arm still short (fig. 95 *TR*).

Experiment R. E. 107. May 10, 1916. Right limb bud to left side, dorsoventral orientation. Pronephros out.

May 11. Rather large area of yolk, posterior to graft, still uncovered.

May 15. Transplanted bud points a little more dorsally and more sharply to side than normal.

May 19. Grafted limb (*TR*) about size of normal, though more pointed and projecting more dorsally and laterally (fig. 96, drawn May 18); divided at end (digits or reduplication?).

May 24. Limb on side of operation still projects a little to side. Normal limb seems to move and transplanted one still stiff (figs. 97 and 98, drawn May 23).

May 28. Motility of operated limb still seems deficient. Third digit distinct (fig. 99, drawn May 29).

June 2. Motility better. Specimen preserved.

Experiment R. E. 116. May 16, 1916. Right limb bud to left side, dorsoventral orientation; pronephros removed.

May 17. Perfectly healed.

May 20. Transplanted bud rather small, points slightly dorso-posteriorly.

May 24. Transplanted bud slightly smaller than normal and points a little more to side. Figure 100, drawn on May 23, shows scarcely any difference between the two buds.

May 28. Transplanted limb (*TR*) practically exact counterpart of normal (figs. 101 and 102, drawn May 29).

May 31. Transplanted limb has third and trace of fourth digit. Function normal.

Experiment R. E. 93. May 19, 1915. Right limb bud to left side, dorsoventral orientation; pronephros mostly removed.

May 21. Well healed; bud smooth and mound-like.

May 25. Transplanted bud points dorsoposteriorly with a secondary bud at anterior border of base.

May 28. Posterior bud (*PR*) is normal in position and orientation, and is even a little longer than the normal limb on the opposite side. The other (anterior) nodule (*DU*) is growing into a limb and also points posteriorly (figs. 103 and 104).

May 30. Posterior bud growing more rapidly than other; digitations plain. Anterior bud sticks out more to side, shows trace of digitations.

June 4. Two hands, each with two digits; some excrescences. Arm a little shorter than normal. The posterior bud is (probably) a left, and its position is approximately normal. The other is mirrored from its dorsal surface. Both hands have faint trace of third digit. Excrescences which seem to involve mainly the skin make an exact interpretation of this case difficult (fig. 105).

C. Superposed transplantations

13. Homopleural, dorsodorsal

Experiment S. E. 3. April 20, 1916. Right limb bud to right side, normal orientation; mesoderm of host torn in posterior part of wound, otherwise intact.

April 21. Well healed; limb bud considerably larger than normal.
April 24. On operated side limb bud larger than normal.
April 26. Operated limb slightly larger, otherwise no difference.
May 2. Operated limb still slightly larger.
May 8. The two limbs exactly alike (fig. 106).

14. Homopleural, dorsoventral

Experiment S. E. 18. April 13, 1917. Right limb bud to right side, inverted; mesoderm all left in.

April 14. Perfectly healed.
April 17. Operated limb bud sticks out more to the side, and is more massive than normal.
April 21. Operated limb double; posterior component more massive, occupies normal position; other is attached to radial border and points dorsoposteriorly; neither has digits.
April 25. Reduplication of hand and part of fore arm; one component is in approximately normal position, the other sticks out to side.
April 30. Limb has grown; relations essentially the same as before.
May 1. Specimen preserved. The main limb projects horizontally to side and is inclined about 30° to the transverse plane; palm is ventral, ulnar border posterior. The reduplicating member with two digits is attached to the radial border of the wrist, pointing anteriorly and laterally (fig. 107).
Experiment S. E. 10. May 26, 1916. Right limb bud to right side inverted; mesoderm of host torn along posterior border of wound and a little tissue lost.

May 27. Perfectly healed.
May 30. Limb on operated side points a little more dorsally than normal. Ventro-anterior to main bud another fairly prominent mass of tissue (like S. E. 9, in which an extra digit developed).
June 4. Limb on operated side considerably smaller than normal, and still points more dorsally; digits just beginning.
June 12. Operated limb still less developed than the unoperated; third and fourth digits beginning to show.
June 15. Practically no difference between the two limbs; the operated one is possibly a little thicker.

15.   Heteropleural, dorsodorsal

Experiment S. E. 12. May 26, 1916. Left limb bud to right side, dorsodorsal orientation; mesoderm of host not injured at all.

May 27.   Perfectly healed.

May 30.   Limb on operated side (*TR*) more massive than normal and points dorsally; on posterior border at base there is a small rounded prominence or bud (figs. 110 and 111, *HOM*, drawn May 31).

June 4.   Main limb (*HET*) is spindling and points laterally, though inclined slightly dorsally and posteriorly; there are three imperfectly marked digits at the tip.   From the bud at the posterior border there has developed a second limb (*HOM*) which has normal posture; it is of considerable size and shows beginning of digitations (fig. 112, drawn June 5).

June 7.   The posterior member is much more massive than the other, which still sticks out to side and shows a very imperfect hand.

June 19.   The posterior member (*HOM*) is practically normal with four digits; it is mobile, though probably there is some extensor weakness of hand.   The anterior member (*HET*), a left (not reversed), is thin and atrophic, the imperfect hand having three digits.   It arises from near the shoulder of the other, the reduplicating plane being approximately radial (fig. 113, drawn June 12).

Experiment S. E. 6.   April 21, 1916.   Right limb bud to left side, dorsodorsal orientation.

April 22.   Perfectly healed.

April 26.   On operated side there are two projections in limb region (fig. 109 *A*).

May 2.   Operated limb not quite so advanced as the normal; distinct spur (fig. 109 *B, S*) on radial border, probably from the anterior of the two prominences.

May 8.   Specimen preserved (fig. 109).   Operated limb (*TR*) not so advanced as normal, digits not so well developed.   The spur (*S*) is attached to the antero-lateral border of arm above elbow and is as large as one of the primary digits.   It is a radial reduplication which has remained abortive.

16.   Heteropleural, dorsoventral

Experiment S. E. 11.   May 26, 1916.   Left limb bud to right side, dorsoventral orientation; mesoderm torn along posterior border of wound; no tissue lost.

May 27.   Perfectly healed.

May 30.   On side of operation a large limb bud points dorsoposteriorly like the normal.   Ventrally another distinct but smaller bud (*DU*) points ventroposteriorly (figs. 114 and 115 drawn May 31).

June 4.   Operated limb (*TR*) longer and further advanced (digits) than normal (*N*).   Otherwise no abnormality (fig. 116, drawn June 5). No trace of ventral bud noted last time.

June 7.   Limb on operated side still a little larger than the other.

June 12.   Still some difference in size (fig. 118); limb normal in form; motility not so good as normal, extensors of hand weak.

June 19. Function of operated limb very much better, almost, if not quite, normal. Specimen preserved. No difference in size of limbs.

D. Transplantation of half-buds

18. Homopleural, dorsodorsal

Experiment H. E. 6. April 12, 1917. Anterior half of limb bud to anterior half, normal orientation (fig. 120, *1*). Operation on right side. Pronephros removed and transplanted.

April 13. Perfectly healed.
April 17. Limb bud on operated side normal.
April 20. Same.
April 30. Same.

19. Homopleural, dorsoventral

Experimental H. E. 31. April 9, 1918. Posterior half of right limb (inverted) in place of anterior right (fig. 120, *6*).

April 10. Wound perfectly healed.
April 15. Operated limb bud smaller than normal, and stands out more sharply from body. It is more distinctly marked off (points) anteriorly.
April 18. Operated bud distinctly more massive (fig. 121, *TR*).
April 21. Hand double, but coalesced to end. Digitations indistinct.
April 30. Practically a normal right limb with an accessory hand growing from back of hand. The preserved specimen shows this to be a case of reduplication mirrored from a dorsal plane slightly inclined to the radial. The reduplicating member consists of second and third digits, (*2′* and *3′*) the former slightly bifurcated. The first (radial) digit is not doubled (fig. 122).

Experiment H. E. 29. April 9, 1918. Ventral half (inverted) of right limb bud in place of dorsal right (fig. 120, *8*). Embryo from which graft was taken, stained in Nile-blue sulphate.

April 10. Healing good. Small round area still uncovered by ectoderm at ventroposterior border of wound.
April 15. Operated bud has grown considerably, pointing dorsally, and slightly anteriorly. Whole free portion of bud covered by stained (grafted) ectoderm.
April 18. Limb on operated side points dorsoposteriorly with reduplicating nodule on posterior border.
April 21. Limb short; double hand.
April 26. Well-developed double hand. Anterior member further developed, sticks out to side; is a left; palm anterior; other a right; dorsoradial reduplication.
May 4. Almost perfectly symmetrical double hand.
May 6. Specimen preserved. Arm as a whole a right, as is the posterior hand. This has three digits and a nodule for fourth, the first

two digits being partly syndactylous. Reduplication is radial. The anterior hand (which is a left) has two long digits and a well-developed third.

Experiment H. E. 2. April 18, 1916. Dorsal half of right limb bud (inverted) in place of ventral right (fig. 120, *7*).

April 19. Healing good, but a groove dividing the limb region horizontally still indicates line of suture.

April 21. Two rather distinct humps on operated side; larger one ventro-anterior.

April 26. Operated limb bud nearly normal, but there is a bud-like projection on the anterolateral border (fig. 123, *S*).

May 1. Transplanted limb larger than normal. The anterior process is now a spur at elbow of the main limb, which is nearly normal.

May 8. Preserved. The operated limb, especially above the elbow is a little thicker, but is otherwise normal. The spur is a nodule just above elbow on radial border (fig. 124).

Experiment H. E. 5. April 12, 1910. Posterior half of right limb bud (inverted) in place of anterior right (fig. 120, *6*).

April 13. Perfectly healed except for minute uncovered area at dorsal border.

April 17. Operated limb bud double; large posterior bud points normally, but is not so large as normal; anteroventral bud is much smaller; it is clear that former is developing out of the remaining half of the limb bud of the host, while the latter is from the graft.

April 20. Both buds have grown; posterior one points more dorso-laterally than normal and shows first beginnings of digitations; anterior bud prominent, rounded, no digitations; the two are separate to base.

April 25. Posterior member is fan-shaped, with three long digits, and beginning of ulnar digits on each margin; anterior member much shorter, with faint indication of digitations.

April 30. Posterior member has a symmetrical fan-shaped five-digitate hand; as a whole it is a right limb, as indicated by elbow bend. Anterior member much shorter and thicker.

May 21. Specimen preserved. The posterior member has a double hand, of which the posterior is a right and the anterior a left. The anterior member is a nearly normal right, with the hand a little twisted and syndactylous second and third digits (fig. 125). This is an anomalous case.

Experiment H. E. 13. May 3, 1917. Posterior half of right limb bud (inverted) in place of anterior half (fig. 120, *6*).

May 4. Perfectly healed.

May 7. Two distinct though small limb buds, one ventro-anterior, the other dorsoposterior.

May 10. Two buds united at base; both project sharply laterally and slightly posteriorly.

May 14. Two limbs; bifurcation in Y-form about at elbow. Anterior member shows two digits; posterior, none.

May 21. Specimen preserved (fig. 126). Hand double; limb (TR) as a whole looks like a right, including the anterior hand (HOM), which has two long digits and a distinct third. The posterior hand ($L ET$) is a left, also with three digits, the first of which is truncated. Reduplication ulnar.

20. Heteropleural, dorsodorsal

Experiment H. R. E. 1. April 18, 1916. Posterior half of right limb bud in place of anterior left (fig. 120, *10*).

April 19. Well healed.

April 21. Two distinct humps on operated side; dorsal one larger, ventro-anterior one quite small.

April 26. Limb on operated side about as large as normal, but projects laterally.

May 1. Reduplication of hand; three digits, fan-like.

May 8. Specimen preserved (fig. 127). Arm somewhat thicker than normal, projects posteriolaterally with hand flexed ventrally. Hand symmetrical, with five digits, of which middle one (*1*) is defective. The posterior half of the hand (HOM) is a left, the anterior (HET) a right.

Experiment H. R. E. 5. April 19, 1916. Dorsal half of right limb bud in place of dorsal left (fig. 120, *11*).

April 20. Perfectly healed.

April 24. Operated limb bud not quite so large as normal, points laterally and slightly anteriorly.

April 29. Operated limb double (fig. 128).

May 8. Specimen preserved (fig. 129). The dorsal (posterior) member (HOM) is a nearly normal left arm; first digit short; third is small, and fourth not visible. The ventral (anterior) member (HET) arises just below elbow, and likewise shows three digits, of which first is short; plane of mirroring is radial.

Experiment H. R. E. 11. April 11, 1917. Anterior half of left limb bud in place of posterior right (fig. 120, *9*). Wound perfectly healed same evening.

April 14. Operated limb bud very large and prominent, position normal.

April 16. Limb bud massive, points dorsolaterally; almost perfect anteroposterior symmetry.

April 23. Limb has now assumed normal position; first two digitations show; a distinct but small spur at elbow on radial border points laterally.

May 4. Operated limb normal; spur much reduced.

May 7. Specimen preserved. Arm normal; spur a mere nodule about middle of upper arm, radiodorsal border (fig. 130).

Experiment H. R. E. 43. April 5, 1918. Anterior half of left limb bud in place of posterior right (fig. 120, *9*).

April 6. Perfectly healed.

April 9. Operated limb a little more massive than normal.

April 11. Limb more massive, rounded, not more distinctly marked off posteriorly than anteriorly when viewed from above. Points dorsally slightly posteriorly in lateral view.

April 14. More massive, but otherwise normal.

April 22. Not quite so long; syndactyly first two digits, otherwise normal.

May 4. Normal except for syndactyly.

Experiment H. R. E. 9. April 11, 1917. Posterior half left limb bud in place of anterior right (fig. 120, *10*). Wound perfectly healed same evening.

April 14. Operated limb bud looks nearly normal. No definite pointing.

April 16. Two rather distinct nodules, the posterior one considerably more prominent.

April 18. Posterior bud smaller than normal, points dorsolaterally. Anterior one not so definite.

April 23. Two entirely separate limbs; anterior one is shorter, points ventroposteriorly; posterior one points straight to side; no digitations.

April 28. Two limbs point posterolaterally, parallel to one another; anterior one thicker, has two digits and beginning of third; apparently a normal, though much smaller, right limb in approximately normal position. Posterior limb thinner, rod-like, with no digits.

May 4. Anterior limb a normal right; posterior, very imperfect, has one long digit and third digit nodule on upper border; elbow bend shows; limb probably also a right (?)

May 12. Anterior limb has good function.

May 21. Specimen preserved. The anterior member is a normal right, somewhat smaller than the unoperated limb. Posterior member has one long and one short digit, and has same general position as the anterior. Total view leaves in uncertainty which is palmar surface (fig. 131). Sections show clearly that the ventrolateral surface is the palm and that the limb is therefore a left.

Experiment H. R. E. 20. May 2, 1917. Dorsal half left limb bud in place of dorsal right (fig. 120, *11*).

May 3. Wound perfectly healed.

May 7. Operated limb about full size, points anteriorly and slightly dorsally.

May 10. Projects straight to side. Slight indication of posterior reduplicating bud from near attachment of limb.

May 14. Main limb points dorsolaterally and slightly posteriorly. The reduplicating bud is attached near base and in part directly to body wall; it has grown considerably, but is still without digits.

May 18. Main (anterior) member sticks out to side, but now points distinctly posteriorly as well, is slender and with two digits. Other limb shorter, but considerably stouter, with faint indication of two digits.

May 27. Anterior member has remained slender, and still has but two digits; distinct elbow bend; general shape indicates it to be a right. The posterior member, practically normal except slightly underdeveloped, is an undoubted right; first two digits partly syndactylous (first short); third is distinct and fourth indicated. Some function in this member.

May 28. Specimen preserved; form of limbs shown in figure 132. Sections show that there are two separate sockets in the shoulder-girdle for the two limbs. The medial surface of both is the palm. Hence both are rights.

21. Heteropleural, dorsoventral

Experiment H. R. E. 36. April 4, 1918. Ventral half left limb bud (inverted) in place of dorsal right (fig. 120, *16*). Pronephros removed.

April 5. Perfectly healed.

April 8. Operated bud a little sharper; points normally (dorso-posteriorly).

April 10. Nearly normal; points slightly more dorsally.

April 13. Normal.

April 16. Normal limb; digitations show.

April 30. Specimen preserved (fig. 133).

Experiment H. R. E. 10. April 11, 1917. Anterior half left limb bud (inverted) in place of anterior right (fig. 120, *13*). Wound completely healed same evening.

April 14. Limb bud operated side nearly normal looking.

April 16. Almost perfectly normal.

April 18. Operated limb normal except slightly larger.

April 23. Reduplication of digits radial side.

April 28. Operated limb a normal right with two digits arising from palmar surface near radial border; arm somewhat thicker than normal.

May 7. Specimen preserved (fig. 134). The first digit is somewhat small; the reduplicating digits (*DU*) are united at base.

special interest are: changes occurring with age, differences due to species and sex, the effects of starvation and hibernation, as well as problems dealing with the various phases of metabolism. Results of such nature obtained for higher forms have contributed much toward the advancement of various theories of growth, senescence, etc. (Child, 1; Mathews, 2; Minot, 3), as well as to our knowledge of changes in organisms which are closely related to age, sex, etc. (Hatai, 4).

The present investigation deals with such problems as the percentage of water during growth, starvation, and 'hibernation' in different species of grasshoppers, and also the rate of metabolism as indicated by determinations of the amounts of carbon dioxide given off by the animals.

MATERIAL AND METHODS

A. Material

Grasshoppers were used in all the following experiments. These animals have been found to be excellent material because of the ease with which they can be obtained, kept under usual laboratory conditions, and handled in experiments. They are sufficiently large to be used individually, and this is of great importance because most of the physiological work heretofore done on insects has been concerned with masses rather than with individual animals.

The following species were used: Melanoplus femur rubrum, Melanoplus differentialis, Dichromorpha viridis, and Chortophaga viridifasciata, and they will be discussed in the order given.

Melanoplus femur rubrum DeGeer, the red-legged locust, is perhaps the most common grasshopper found throughout the entire United States. Its general life-history is practically typical; eggs are laid in the late summer and early fall and remain over winter; nymphs hatch in early summer, and by the last of July and early part of August, in the vicinity of Philadelphia, adults are found. It occurs in rather large numbers throughout the entire summer. The average length of the body of adult

males is 23.5 mm., and of females, 24.5 mm.; average weights are: adult males, 0.20 to 0.40 gram; females, 0.25 to 0.65 gram. Nymphs range in weight up to a maximum of 0.35 gram.[1]

Melanoplus differentialis Uhler, the largest grasshopper found in this vicinity, closely parallels Melanoplus f. rubrum in life-history. In length adult males measure 39 mm., and females, 41 mm. Adult males weigh 0.7 to 1.3 grams, and females, 1.3 to 2.8 grams.

Dichromorpha viridis Scudder has a general life-cycle similar to the above-described species. However, it is not as active an animal and occurs in open wet places. Differences in size between adult males and females are marked. Adult males measure 18.75 mm., and females, 27.0 mm. In weight adult males range from 0.15 to 0.20 gram, and females, from 0.15 to 0.55 gram.

Chortophaga viridifasciata DeGeer is quite different in life-cycle from the above-mentioned species. Eggs are laid in late spring and early summer; these hatch in later summer and early fall; the nymphs live throughout the winter, and in spring grow rapidly, and become adults by early summer. Two-thirds of their active life, in contrast with other species, is spent as nymphs and approximately one-third as adults. Two well-marked varieties occur, a green form (virginaria Fab.) and a brown form (infuscata Harris). Most females are green and males brown, but some are found of each sex in either color, and as a matter of fact, when green animals are put at a constant temperature of 38°C. they turn brown in a very short time. Adult males measure 25 mm. and weigh 0.10 to 0.20 gram; females measure 30 mm. and weigh 0.15 to 0.45 gram.

For further descriptions of the above species reference is made to standard text-books on entomology and to the works of Morse (6) and Lugger (5).

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[1] Average dimensions of animals used are taken from Lugger (5), while body weights have been determined by the author.

B. Methods

The following general description of methods applies to all experiments and any further details will be given in describing individual cases.

All animals were caught in the vicinity of Philadelphia during the summer and fall of 1919–1920, brought into the laboratory, where they were kept in large screened insect cages, designated as stock cages. They remained in these cages for at least a day under the usual laboratory conditions, and were fed during this time on grass. Inasmuch as grasshoppers normally consume a great deal, those kept in the laboratory for a day ate large amounts of the grass, and upon examination the alimentary canal was found to be filled, thus insuring uniformity as to initial amounts of food. General laboratory conditions remained constant throughout the experiments, and any slight temperature changes, usually occurring at this particular season of the year, are noted in data following.

Animals were separately weighed in a small covered beaker on a rather delicate balance, determinations being made to four places of decimals. After weighing they were marked by gluing a small numbered tag on the pronotum, which mark could easily be removed and again attached, thus avoiding confusion in keeping accurate records of individuals. After initial weighing they were kept, in groups of five to ten, in small wire insect cages.

In determining water content, individuals were killed with chloroform, opened by a longitudinal slit through the abdomen, and then put in an oven at 95° to 97°C. and left there for a period of one week. This was found to be more than ample time for complete desiccation.

Carbon-dioxide determinations were made by the barium hydrate titration method of Lund (7), using single animals, and each determination extending over a period of thirty minutes to one hour. In suspending individuals in the respiration bottle they were carefully tied around the prothorax by means of a fine silk thread. This was found to cause them little incon-

venience if properly adjusted, and if it was kept sufficiently short, they did not move about to any appreciable extent but rested upon the sides of the bottle. Experiments in which the animal was confined in a small wire cage, just large enough to accommodate it and not allowing body movements, gave results quite similar to those in which the animal was suspended by the thread. In any such experiments, however, it is quite impossible to entirely eliminate movements by the individual, but by careful handling and manipulation they can be greatly lessened, and comparable, if not accurate, results obtained. In the following experiments emphasis is laid upon relative rather than upon absolute amounts of carbon dioxide given off.

Acknowledgment is made to Prof. C. E. McClung for the original suggestion of work on the physiology of the grasshopper, and to Prof. M. H. Jacobs I am deeply indebted for constant advice and criticism during the course of the work. To the members of the Zoological Department of the University of Pennsylvania I am also greatly indebted for generous help and criticisms.

<div align="center">OBSERVATIONS</div>

<div align="center">A. Water content</div>

The physiological importance of water to organisms is too well known to require special discussion. In recent years, many determinations of water content have been made on different organisms, and on the same organisms under different conditions, and the results have thrown much light on such questions of biological interest as the cause of certain tropic responses (Breitenbecher, 8), the nature of the process of senescence (Hatai, 4), the question of the influence of different foods (Babcock, 9), etc. Determinations of this sort have been made mostly on higher forms, and no such observations appear to be available for grasshoppers, which I have, therefore, studied rather extensively.

a. *Relation between body weight and water content.* Table 1 gives the body weights and water contents of 981 individuals,

comprising nymphs and adults of Melanoplus f. rubrum and adults of Dichromorpha viridis and Melanoplus differentialis. It is evident from an examination of this table that considerable variation in the body weights for the different species exists. These can be explained by a consideration of the differences due to species, age, and random variations. It will be noted, for instance, that of the species studied, Melanoplus differentialis is by far the heaviest, reaching a maximum of 2.9 grams. Range in weight for Melanoplus f. rubrum is of interest, since weights for both nymphs and adults are given, and these show that as the animals become adults there is a progressive increase in body weight up to a maximum for the species. Males never reach the same maximum weights as females, and this is not due primarily to development of masses of eggs by the older females, since in nymphs this relation also holds. Consequently any comparison between males and females of the same weights will not necessarily be between those of the same age. Different conditions, such as food supply, development of reproductive elements, etc., modify the maximum weights of animals, causing some variation among individuals of the same age as shown for Melanoplus differentialis, where the animals are of approximately the same age and show rather large variations in body weights.

Closely related to these differences in body weights of the animals are the changes occurring in the percentages of water. With increasing body weight and age, a progressive diminution in the relative water content takes place, as shown especially by Melanoplus f. rubrum, where nymphs have an average maximum of 77.6 per cent and adults an average minimum of 72 per cent. That this diminution in water content is related to age, and not to body weight, is shown by a comparison of these results with those for Melanoplus differentialis, where the animals are of the same age, but differ in body weights, and have approximately the same percentage of water. A similar result has been found by Hatai (4), for the albino rat, in which the percentage of water during the life-span decreases from 87.2 per cent to 65.3 per cent and bears no relation to body weight, but depends primarily upon the age of the animal. It will also

be noted that the average percentage of water for the adult males of Melanoplus f. rubrum tends to be slightly higher than that for the females, and that for Dichromorpha viridis the reverse relation is found.

TABLE 1

Showing the percentage of water in normal grasshoppers of different species. Figures in ( ) giving the number of animals used

BODY WEIGHT	PERCENTAGE OF WATER							
	Melanophus f. rubrum (nymphs)		Melanophus f. rubrum (adults)		Dichromorpha viridis (adults)		Melanophus differentialis (adults)	
	♂	♀	♂	♀	♂	♀	♂	♀
grams								
0.10–0.15	76.7 (8)	78.5 (11)			69.8 (34)	75.1 (25)		
0.15–0.20	75.0 (10)	77.9 (14)	74.9 (7)		64.9 (15)	74.3 (15)		
0.20–0.25	74.8 (13)	74.1 (15)	74.2 (19)	76.0 (25)		74.3 (40)		
0.25–0.30		73.9 (10)	73.6 (32)	74.1 (40)		73.3 (42)		
0.30–0.35		74.4 (12)	73.4 (47)	74.5 (45)		71.8 (50)		
0.35–0.40			73.9 (19)	72.5 (23)		68.4 (57)		
0.40–0.45				70.8 (12)		67.9 (40)		
0.45–0.50				73.1 (14)		65.4 (20)		
0.50–0.55				71.2 (27)		66.7 (18)		
0.55–0.60				71.1 (13)				
0.60–0.65				70.3 (18)				
0.90–1.10							69.3 (15)	
1.10–1.30							69.6 (11)	
1.30–1.50								69.7 (10)
1.50–1.70								66.8 (12)
1.70–1.90								71.0 (25)
1.90–2.10								69.2 (18)
2.10–2.30								69.0 (10)
2.30–2.50								69.4 (34)
2.50–2.70								69.9 (27)
2.70–2.90								69.3 (19)
Average.	75.5 (41)	75.7 (62)	74.0 (124)	72.6 (217)	67.3 (49)	70.8 (307)	69.4 (26)	68.0 (155)

b. Specific differences. It will be noted from table 1 that the average percentage of water differs for the various species studied. For example, Melanoplus f. rubrum has an average water content of 74.4 per cent, Dichromorpha viridis 69 per cent, and Melanoplus differentialis 68.9 per cent. These averages (except

those for Melanoplus differentialis) are based upon individuals taken during the entire summer and hence represent values for both young and old animals and can be considered as fair averages for the species. That such an average must be based upon the water contents of both young and old individuals is quite evident, since the preceding section shows that the percentage of water of an animal decreases with increasing age. It is of some interest to mention here that in another species of grasshopper, Chortophaga viridifasciata, the percentage of water during the winter months drops to a minimum of 65 per cent, while in the same animal when growing the water content is raised to 75 per cent. Since the percentage of water of an animal is thus dependent upon and so easily modified by external factors, such as temperature, moisture, etc., it is difficult to get data to show that definite species differences in water content exist. The present results for Melanoplus f. rubrum and Dichromorpha viridis, where the animals were under the same conditions, seem to show, however, that such a condition might occur. Babcock (9), in discussing 'metabolic water,' concludes that the water content of insects depends largely upon the nature of the food eaten, but does not show that those living upon the same foods may have different percentages of water.

c. *Changes during the normal life-cycle.* Some changes in the percentage of water during the life-cycle of grasshoppers have been shown in a preceding section. Chortophaga viridifasciata, however, because of its peculiar habit of living as a nymph during the winter and 'hibernating,' affords opportunity for further studying the various changes in water content which normally occur during such a period.

Table 2 gives results obtained for 565 specimens and shows that during this period the percentage of water falls from 72 per cent to 65 per cent. This minimum of 65 per cent is perhaps as far as desiccation can be carried without injurious results, and is a factor of some importance in the economy of the organism. Somewhat similar rhythmic variations in the water content of animals are shown for the frog by Donaldson (10), and for the potato-beetle by Breitenbecher (8). These 'hibernating' animals

with low percentages of water, left at 38°C. for a period of three
weeks and fed grass, grow and become adults, and the various
changes in the water content during this period are easily followed.

Table 3 selected from several such experiments shows that a
growing animal has the maximum percentage of water (75 per
cent), and that a progressive decrease in water content with
age and increasing body weight, to a minimum of 65 per cent,

TABLE 2

*Showing the percentage of water and solids of Chortophaga viridifasciata taken at
different periods of the year*

DATE	TEMPERATURE	PERCENTAGE OF		NUMBER OF ANIMALS
		Water	Solids	
	°C.			
October 10, 1919.	22–25	72.0	28.0	50
October 11, 1919.	22–25	72.0	28.0	35
November 4, 1919.	13.0	69.4	30.6	25
November 5, 1919.	8.0	69.4	30.6	15
November 13, 1919.	10.0	66.5	33.5	17
November 14, 1919.	4.5	66.1	33.9	43
November 18, 1919.	7.0	69.5	30.5	14
November 19, 1919.	7.5	67.3	32.7	60
November 23, 1919.	11.0	67.3	32.7	47
November 26, 1919.	9.0	65.7	34.3	62
December 3, 1919.	0	65.2	34.8	50
December 8, 1919.	5.0	65.2	34.8	34
December 16, 1919.	0	67.4	32.6	19
December 17, 1919.	0	66.5	33.5	38
December 22, 1919.	0	62.8	37.2	15
January 4, 1920.	0	65.0	35.0	41

takes place.   Figure 1 gives graphically and in more detail,
similar results taken from many experiments which show the
water content and weight relations of animals during the period
from October, 1919, to January, 1920.   The results of two
experiments at different intervals, giving the effects of a constant
temperature of 38°C., are also indicated.

From this figure it is to be noted that during this four-month
period, rather marked changes in the water content of animals
take place, while body weights undergo only slight and gradual

increases. With the approach of cold weather the animal begins gradually to lose water, and with sudden decrease in temperatures rather marked drops, to a minimum of 65 per cent,

TABLE 3

Showing the changes in water content of Chortophaga viridifasciata during different stages of its life-cycle

WEIGHT OF ANIMAL	STAGE OF GROWTH	CONDITION	PER CENT OF WATER
grams			
0.0598	Nymph	Growing	74.9
0.0648	Nymph	Growing	76.8
0.0749	Nymph	Growing	73.2
0.0800	Nymph	Growing	75.2
0.0895	Nymph	Growing	77.6
0.1100	Nymph	Growing	77.8
0.1700	Nymph	Growing	73.8
0.2498	Nymph	Growing	74.1
0.0700	Nymph	Hlbernating	64.8
0.0750	Nymph	Hibernating	60.6
0.0750	Nymph	Hibernating	65.4
0.0725	Nymph	Hibernating	65.6
0.0848	Nymph	Hibernating	64.7
0.0840	Nymph	Hibernating	66.7
0.0885	Nymph	Hibernating	66.0
0.1020	Nymph	Hibernating	65.7
0.2780	Adult	Growing	76.5
0.3850	Adult	Growing	73.4
0.3715	Adult	Growing	75.3
0.3150	Adult	Growing	74.7
0.4130	Adult	Growing	73.4
0.4230	Adult	Old	67.0
0.1350	Adult	Old	61.5
0.2640	Adult	Old	68.6
0.3020	Adult	Old	69.3
0.2480	Adult	Old	63.2
0.3970	Adult	Old	61.6
0.3630	Adult	Old	61.5
0.4570	Adult	Old	64.9

occur. The water content then remains at this minimum during the remainder of cold weather. Despite this preparation for the winter by a falling off in the percentage of water, the process seems one quite easily changed at any period by exposure to a

higher temperature of 38°C. The two experiments represented
show results typical for many others obtained at different inter-
vals during this four-month period. It will be noted that a
marked and steady increase in body weight takes place, until
at the end of approximately three weeks, the maximum for the
species is attained. The percentage of water, on the other hand,
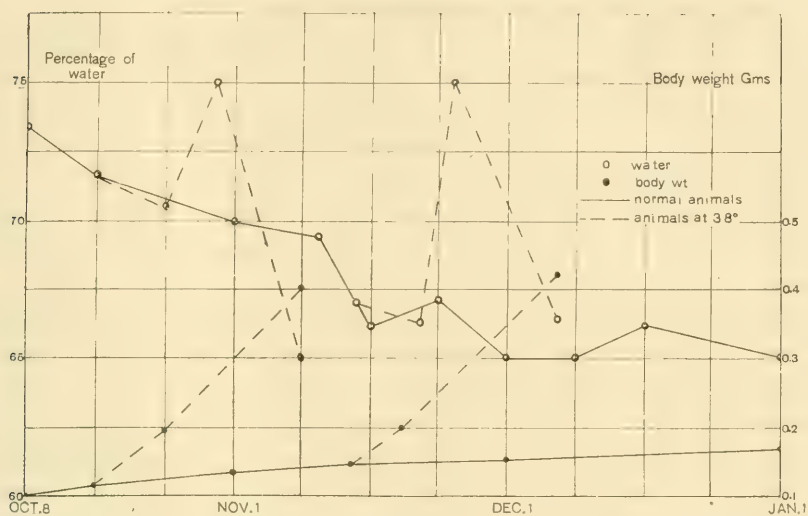
Fig. 1 Curves show the water content and weight relations of Chortophaga
viridifasciata, during four months, from October 8, 1919, to January 1, 1920, out
of doors, and also the effects of 38°C. on these relations in 'hibernating' nymphs.
Abscissas, time in weeks indicated. Ordinates, at the left, the percentage of
water. Ordinates, at the right, body weight in grams. For further explanation
see description in text.

undergoes rather striking and regular changes. A slight decrease,
followed by a rapid increase to a maximum of 75 per cent and
accompanied by active growth, and a correspondingly rapid
decrease, closely connected with the later stage of the animals'
life, occur. Such a result strikingly confirms conclusions arrived
at in an earlier section in which such differences in water content
were shown to be correlated with the age and not the body weight
of an animal.

d. Effects of temperature. As pointed out in the preceding section, raising the temperature to 38°C. causes marked changes in the water content of 'hibernating' individuals. That such results can be obtained at other temperatures is shown from a series of experiments in which 'hibernating' nymphs were kept at 9°, 23°, and 38°C., respectively, for periods of three weeks and fed grass. Progressive increases in the percentage gains of water, body weight, and solids take place in all three series, being highest, however, at 38°; while with nothing to eat at these temperatures the animals die, thus showing that during the winter a real hibernation can hardly be supposed to take place, since it is generally understood that hibernating animals require no food other than that already stored in the body.

e. Effects of relative humidity of the air. 'Hibernating' nymphs were put into small cages covered with wire gauze and the cages were then put into, 1) sealed jars containing wet sand and filter-paper, 2) sealed jars with dry sand, and, 3) a desiccator containing calcium chloride, and kept at temperatures from 4° to 38°C. The animals were not in contact with the sand or wet filter-paper, and hence any increase in weight or water content cannot be attributed to imbibed water. Nothing was given the animals to eat. Table 4 shows the percentage loss in weight, water, and solids for ninety-five individuals treated in such a manner.

In general it is found that in the jars with dry sand and in the desiccators the animals lose weight and water, the losses being highest at 38°. Losses in water are relatively higher than those in body weight. In the jars with the wet sand, on the other hand, marked increases in body weight and water result. At 4°C., however, a slight loss in weight (1.4 per cent) is noted, but an increase in water of 3.5 per cent takes place. Such a slight absorption of water at this lower temperature further shows how the organisms are protected during winter, preventing freezing and possible destruction. It is evident from these results that 'hibernating' nymphs are able to take up water directly from the surrounding medium. Breitenbecher (8) finds a similar condition in the potato-beetle. It is of interest to note, too, that old individuals with low percentages of water are unable

to readjust their water relations when exposed to decreased temperatures. The animal's ability to regulate its moisture content seems to be connected with its ability to grow and withstand adverse conditions.

TABLE 4

Showing the percentage changes in weight, water, and solids in 'hibernating' Chorto-phaga viridifasciata when exposed to differences in relative humidity

INITIAL WEIGHT	PERCENTAGE OF				TIME	NUM-BER OF ANI-MALS	TEM-PERA-TURE	REMARKS
	Water	Change in weight	Change in water	Change in solids				
grams					hours		°C.	
0.4130	66.4	−2.8	−3.7	−0.7	48	5	4	In cage with sand
0.4640	66.2	−1.7	−3.3	+1.6	48	5	4	In jar with sand
0.2950	60.5	−10.4	−23.9	+16.1	48	5	15	In jar with sand
0.3250	61.3	−8.6	−15.5	+5.0	24	5	23	In jar with sand
0.3560	59.5	−14.6	−23.6	+2.1	48	5	23	In jar with sand
0.4150	60.5	−24.9	−41.6	+7.1	24	5	38	In jar with sand
0.4180	65.4	−2.8	−5.7	+1.7	48	5	4	In desiccator
0.3810	60.5	−9.9	−17.7	+7.0	48	5	15	In desiccator
0.3695	60.0	−7.3	−16.2	+10.5	24	5	23	In desiccator
0.4315	60.1	−14.7	−23.5	+2.4	48	5	23	In desiccator
0.3730	59.9	−24.1	−34.0	−4.4	24	5	38	In desiccator
0.5170	68.1	−1.4	+3.5	−10.7	48	5	4	In jar with wet sand
0.4230	64.8	+3.5	+0.5	+9.5	48	5	15	In jar with wet sand
0.3830	62.5	+0.5	+0.3	+0.7	24	5	23	In jar with wet sand
0.5810	71.6	+14.3	+22.6	−2.3	48	5	23	In jar with wet sand
0.3950	73.0	+15.5	+28.0	−9.0	24	5	38	In jar with wet sand
0.4345*	64.6	−14.8	−18.0	−8.1	24	5	38	In jar with wet sand
0.4660*	64.4	−14.8	−18.7	−6.6	24	5	38	In desiccator
0.4840*	61.9	−3.7	+1.9	−15.6	24	5	38	In jar with wet sand

$+$ = gain, $-$ = loss.   * = animals previously kept at 38°C.

B. Effects of starvation

Before dealing with the direct effects of starvation on water content, solids, and body weight of these animals, some points of general interest deserve consideration, one of which is length of the starvation period. It is found that adults of Melanoplus f. rubrum endure complete starvation approximately 73 hours, while with water, but no food, they live as long as 144 hours

with a loss of 30 to 35 per cent in body weight. On the other hand, Melanoplus differentialis, a larger species, survives complete starvation approximately 96 hours, and with water alone, about 172 hours with a loss in body weight of 20 to 25 per cent. Hibernating nymphs of Chortophaga with nothing to eat, at temperatures from 0° to 9°C., can survive only a little more than two weeks; at 23° they live about one week, and at 38° only three to four days. The maximum loss in weight up to death ranges from 20 to 25 per cent. Such a short survival period for the grasshopper is in marked contrast with that found for certain insects and related forms. Dufour (11) for example, kept bedbugs for a year without food, while Riley and Johannsen (12) cite examples where certain ticks were kept for over three years with nothing to eat.

The changes brought about by starvation in the body weight, water, and solids of the grasshopper are rather striking as the following results show. In all experiments adult animals were used and were weighed at twenty-four-hour intervals at the same time each day. The number for Melanoplus f. rubrum is 250, and for Melanoplus differentialis, 75. Room temperature during the experiments remained at 22° to 25°C. Table 5 gives the percentage losses in body weight, water, and solids for different-sized individuals and for the two sexes of Melanoplus f. rubrum, during seventy-two hours of starvation, with and without water. Figure 2, taken from this table, shows the average losses in body weight and water, and figure 3 gives the average percentage loss in weight and also the average percentage loss in weight per day or the rate of loss, with water alone, for Melanoplus differentialis.

From an examination of these data we find that losses in body weight during starvation are marked, and that they increase progressively as starvation proceeds up to a maximum for the species. The rate of loss, indicated in figure 3, is greatest, however, during the first forty-eight hours and diminishes subsequently up to the end of the experiment. Losses in water, as shown in figure 2, are always relatively greater than those in weight, and maintain this same general relation throughout
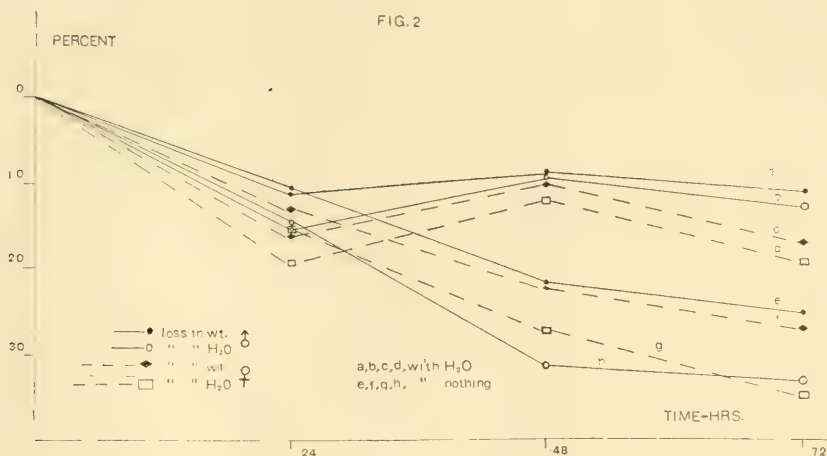
Fig. 2 Curves show the average percentage losses in body weight and water during starvation, with and without water, for both sexes of Melanoplus f. rubrum. Abscissas represent period of starvation in hours indicated by numbers. Ordinates represent percentage of losses occurring during starvation. See table 5.
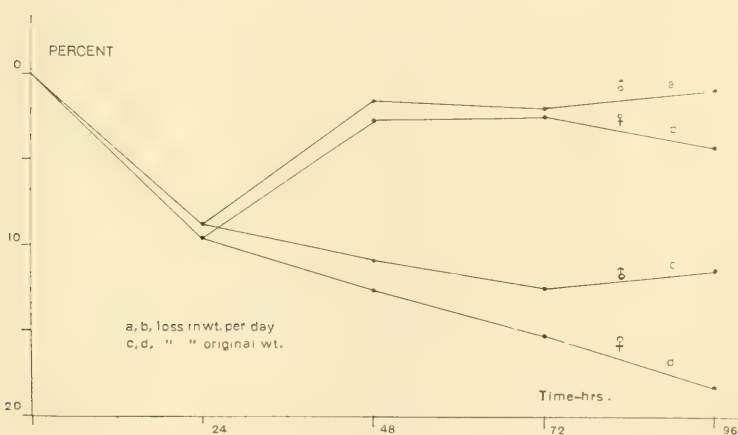
Fig. 3 Curves show the average percentage loss in body weight per day and the average percentage loss of original weight during starvation, with water, for both sexes of Melanoplus differentialis. Abscissas represent period of starvation in hours indicated by numbers. Ordinates represent percentage losses occurring during starvation.

TABLE 5

Showing the water content and the percentage losses in body weight, water, and solids in Melanoplus f. rubrum during starvation. Top figures denoting losses without water and lower ones those with water

INITIAL BODY WEIGHT	TIME	PERCENTAGE OF							
		Water		Loss in wt.		Loss in H_2O		Loss in solids	
		♂	♀	♂	♀	♂	♀	♂	♀
grams	*hours*								
0.20–0.25	24	73.9	73.8	7.2	2.2	8.0	5.2	4.6	+6.6
0.25–0.30	24	71.3		10.9		14.0		2.5	
		69.5	76.7	13.1	12.8	18.1	9.4	+1.2	21.7
	48	63.5		22.6		33.4		+7.5	
		76.3		8.7		5.4		17.7	
	72	65.7		28.3		36.3		6.3	
		74.1		9.2		8.5		11.3	
0.30–0.35	24	70.0	73.8	13.4	13.3	17.5	14.1	2.1	11.2
		69.1		10.9		14.7		+5.3	
	48	65.9	69.2	20.6	20.2	28.7	26.0	+2.1	3.3
		72.5		8.7		9.7		5.3	
	72	68.8		22.5		27.1		9.6	
		70.9		11.7		14.7		3.2	
0.35–0.40	24	67.7	72.6	13.4	15.8	20.9	15.5	+7.6	18.1
		70.1		10.5		14.7		+1.9	
	48	64.4	70.8	22.8	25.7	32.7	27.5	+5.7	20.9
		71.2	75.1	8.6	2.5	11.8	+1.0	+0.9	11.8
	72	63.4	66.1	25.2	24.6	35.8	31.0	+4.8	7.2
		70.5	74.2	11.6	15.6	15.5	13.4	0.0	20.9
0.40–0.45	24		71.9		13.7		12.5		16.7
			68.2		11.9		15.3		3.8
	48		67.5		23.0		26.6		14.5
			71.3		9.4		8.7		10.6
	72		66.2		24.8		29.7		12.2
			68.4		18.6		27.8		19.0
0.45–0.50	24		67.2		17.0		23.8		+1.4
			66.1		13.7		22.1		+8.9
	48		65.6		24.6		32.5		2.9
			69.5		12.4		16.6		0.7
	72		64.7		26.4		34.9		2.9
			66.5		15.3		10.3		+23.0

TABLE 5—*Continued*

INITIAL BODY WEIGHT	TIME	Water		Loss in wt.		Loss in H$_2$O		Loss in solids	
		♂	♀	♂	♀	♂	♀	♂	♀
grams	*hours*								
	24		66.8		13.3		18.6		0.0
			67.4		18.8		23.2		7.5
0.50–0.55	48		64.6		20.7		28.0		2.5
			70.4		15.6		16.5		13.2
	72		61.5		33.9		42.8		11.3
			71.1		18.0		18.1		17.7
	24		66.0		13.9		20.1		+1.7
			67.6		17.9		22.0		7.3
0.55–0.60	48		64.7		18.2		25.5		0.0
			68.2		11.8		15.4		2.8
	72		63.5		26.9		34.6		7.5
			65.8		17.0		23.1		1.7
	24		69.4		15.6		16.6		12.9
			66.7		21.2		25.1		11.9
0.60–0.65	48		65.9		22.7		27.5		11.3
			68.1		9.6		12.4		2.5
	72		64.5		25.6		31.7		10.8
			66.4		19.7		24.0		9.3

starvation. Losses in solids, however, are invariably lower than those in body weight and water. This shows that starvation in the grasshopper results in a rapid loss in water which has a decidedly quick and fatal effect. In striking contrast to such a condition, Hatai (*13*), with medusae, and Morgulis (*14*), with salamanders, find that during starvation the water content is increased rather than decreased, but it must be remembered that in these cases we are dealing with aquatic forms. Table 5, arranged according to body weight, shows that considerable variation in the losses for different-sized individuals exists, but that after the first twenty-four hours of starvation, larger animals tend to suffer the greater relative losses. This is perhaps due to the fact that the lighter individuals are still growing, and as pointed out by Donaldson (*15*) in experiments with rats, the loss of water in the nervous system of underfed individuals is

decidedly less for growing animals, and in growth such losses are markedly more fatal than when growth has ceased. It is also of interest to note that the average losses in body weight and water for males are lower than those for females.

The general effects of starvation, with and without water, are more graphically shown in figure 2, where some of the data from table 5 are represented in the form of curves. It is quite evident from these that grasshoppers must normally require water, and that any condition which deprives them of it results in marked losses to the animal, which rapidly become fatal. No results on 'metabolic water' (Babcock, 9) are available, but it appears that in the grasshopper there is present little of the power shown by clothes moths, etc., of maintaining the proper degree of moisture in the body tissues from water resulting from the oxidation of the organic matter comprising the food and tissues of the animal.

C. Carbon-dioxide output

The respiratory exchange of animals is of physiological significance, since it gives quantitative evidence of the metabolic processes taking place in the organism. Measurements are made either of the oxygen consumption or the carbon-dioxide output, and at present methods for the detection of the latter quantity have been greatly improved and are especially favorable for work on lower forms, such as insects. The factor of greatest importance in such determinations, however, is the functional activity of the animal. In the organism as a whole, functional activity can be reduced only to a minimum, and in those animals, like insects, where narcotization is impossible, only approximations to this can be obtained.

From the results of various investigators, it is of interest to note that the respiratory rates for insects are considerably higher than those for other animals. For example, Vernon (16), finds that a cockroach, weighing 0.0007 kilogram, gives off 0.470 gram of carbon dioxide per kilogram per hour, while a frog, weighing 0.004 kilogram, gives off only 0.140 gram. Smaller and younger individuals of different species tend to have the

higher respiratory rates. It is of importance, however, to mention here that most of the work heretofore done on insects has been concerned with masses rather than with individuals, and that little consideration has been given to results obtained for different species of the same general group, for different sexes, and for animals of different ages. The present discussion deals with the carbon-dioxide output of individual animals of different
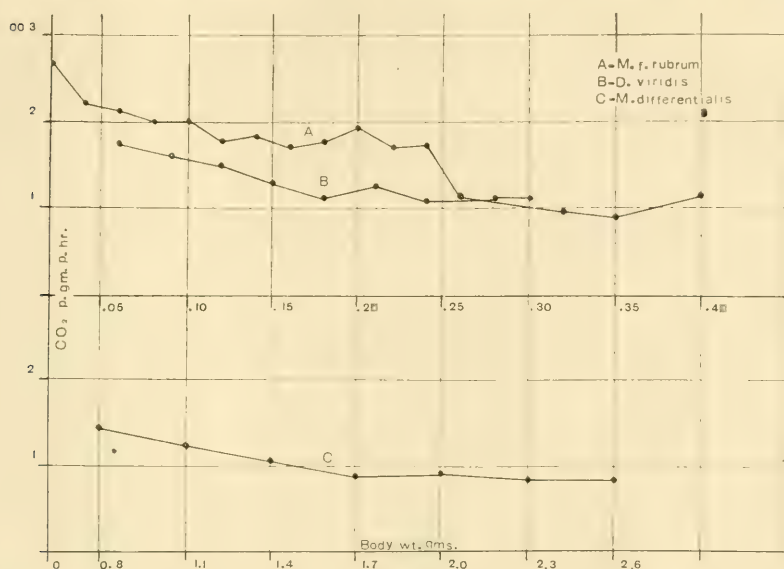
Fig. 4   Curves show the rate of CO_2 output per gram per hour for nymphs of Melanoplus f. rubrum and Dichromorpha viridis and for adults of Melanoplus differentialis. Abscissas represent body weights in grams. Ordinates represent rates of CO_2 output in grams CO_2 per gram total body weight per hour, for the three species. For further explanation see text.

species, different ages, and of different sexes, under normal as well as experimental conditions. The following species of grasshoppers were studied: nymphs of Melanoplus f. rubrum and Dichromorpha viridis and adults of Melanoplus differentialis.

a. Carbon-dioxide output of normal animals. The number of individuals studied is, for nymphs of Melanoplus f. rubrum, 350; for nymphs of Dichromorpha viridis 300, and for adults of Melanoplus differentialis, 85. Figure 4 shows the average rates of carbon-dioxide output per gram per hour for these animals.

An examination of this figure shows several interesting facts, the most striking of which is that a difference in rate of CO_2 output is noted between the three species. That such a difference is not due to body weight is shown by a comparison of the respiratory rates of nymphs of Melanoplus f. rubrum and Dichromorpha viridis, which are of approximately the same weights. The most plausible explanation of this fact seems to be that this difference corresponds closely with the mode of life of the two species, Melanoplus f. rubrum being a very active animal, while Dichromorpha viridis is a relatively sluggish one. A point of further interest is that the rate of CO_2 output is higher for lighter animals and decreases progressively as the animals increase in body weight. As it has already been pointed out that differences in body weight, especially in nymphs, are closely correlated with differences in age, we are led to assume that younger individuals have the higher rate of respiratory output. Like results are also found for other species. Since differences in body weight between males and females exist, the question naturally follows as to whether similar differences in the rate of CO_2 output are found. Figure 6 shows that males tend to have the higher rate. The animals of the two sexes, in this case, are of approximately the same age, and differences in weight, as shown in a previous section, are due mostly to eggs in the female. Whether any fundamental difference in rate of respiratory exchange exists between the two sexes is somewhat doubtful, but such differences are reported for other animals, including man (Benedict and Emmes, 17).

b. *Rate of output.* Much evidence has been accumulated concerning higher forms and man to show that smaller individuals have a greater respiratory exchange per unit of weight than larger ones, and that respiratory exchange is proportional to the area of the surface of the body (Rubner, 18). For lower forms few such observations exist, Child (19), and Allen (20), for example, with Planaria, find that respiratory exchange decreases as the size of the worm increases, but give no calculations showing any possible surface relations. Krogh (21), in summarizing work done on lower forms, finds that results are conflicting, and

concludes that no reason exists for assuming a surface relation to hold.

As already pointed out for the grasshopper, smaller individuals have per unit of weight a greater CO_2 output than larger ones. And since the area of the surface of an animal is usually estimated from the body weight by means of the formula of Meeh (22), based on the law that surfaces of similar solids are proportional to the two-thirds power of volume, it is of some interest to see in how far the rate of CO_2 output of the grasshopper can be thus expressed. In the following table are given a few examples of the ratio of CO_2 output to body weight and to the two-thirds power of the weight, respectively.

WEIGHT OF ANIMAL	CO2 ACCORDING TO BODY WEIGHT	CO2 ACCORDING TO THE TWO-THIRDS POWER OF THE BODY WEIGHT
grams		
2.16	0.001000	0.001290
1.35	0.001037	0.001204
1.11	0.001174	0.001224
1.08	0.001186	0.001226
1.05	0.001215	0.001236
1.01	0.001219	0.001232
1.01	0.001263	0.001276
0.94	0.001310	0.001282

It is evident that the more constant values are obtained by using the two-thirds power of the weight, and so far as the results here reported are concerned, the conclusion might reasonably be drawn, that the surface law holds for grasshoppers as well as for mammals. But in view of the complex nature of the problem, more extensive data will be necessary before this relation can be considered as definitely established.

c. Effects of temperature. The influence of temperature on the respiratory exchange is a somewhat disputed question because comparatively few observations are made under standard conditions. Krogh (21), however, in summarizing the work of various investigators, points out that different animals respond in different ways, but in general, with cold-blooded forms, increased temperatures cause increased respiratory rates, while

with warm-blooded animals, the reverse is the case. The effects
of temperature on the respiratory exchange of insects are espe-
cially marked and in general agree with results for other cold-
blooded forms. But since most of these results are based upon
masses rather than individuals, it has seemed desirable to show
the effects of temperature upon the respiratory exchange of
individual animals. Three hundred and fifty nymphs of Melano-
plus f. rubrum and three hundred of Dichromorpha viridis were
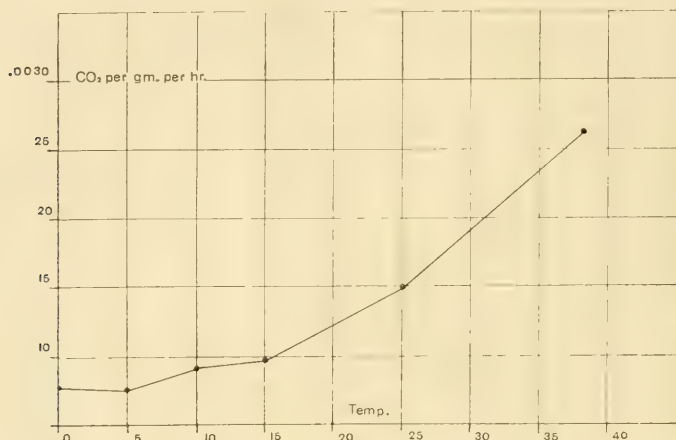
Fig. 5 Showing effects of various temperatures on the CO_2 output of nymphs
of Melanoplus f. rubrum and Dichromorpha viridis. Abscissas represent tem-
peratures in degrees centigrade as indicated by the numbers. Ordinates repre-
sent rate of CO_2 output in grams CO_2 per gram total body weight per hour. For
further explanation see text.

used. Figure 5, plotted from results, shows the average rate of
CO_2 output at various temperatures, ranging from 0° to 38°C.
Examination of this figure shows that in general grasshoppers
respond to temperature changes as do other cold-blooded forms;
that is, increased temperatures cause increased respiratory rates.
At 38° the rate of CO_2 output is highest and in the interval
from 0° to 15° it is nearly constant. This deviation from a
regular increase from 0° to 15° is difficult to explain and perhaps
is due to the imperfect control over conditions, such as body
movements, etc. However, careful observation during the course

of the experiments seemed to show no appreciable differences in body movements at the various temperatures. It is interesting to note to what extent these variations in respiratory rates are directly influenced by the different temperatures, and if a constant temperature coefficient, similar to that for other biological processes and chemical reactions, exists. As is well known, temperature influences on the velocity of certain chemical reactions can be satisfactorily expressed by the rule of van't Hoff, that for an increase in temperature of 10°C., the rate is approximately doubled or trebled, i.e., there is a constant ratio, Q_{10}, of 3-2 for the rates at temperatures separated by an interval of 10°C. Applied to the present results, Q_{10}, varies considerably, increasing with increasing temperatures, and is highest at 15° to 25° (1.5), and lowest at 0° to 10° (1.1). These figures, it will be noted, are somewhat lower than values obtained for chemical reactions. Values obtained for other biological processes are varied as the following examples show. Krogh (21), in experiments on the effects of temperature on the respiratory exchange of the chrysalids of the meal-worm, finds that Q_{10} for temperatures from 10° to 30°C., varies from 5.7 to 2.0, being highest for lower temperatures. Respiration in seedlings from 0° to 40°C. has a value for Q_{10} of 3 − 2, (Clausen, 23) and in a leaf 2.4 − 1.8, (G. L. Matthaei, 24). Here, too, the values for Q_{10} are highest at lower temperatures and decrease as the temperature increases.

There is no fundamental reason why the respiratory exchange of an animal should follow the rule of van't Hoff, since we are dealing, not with a single chemical reaction, but rather with a group of reactions, most complex in nature. Why the temperature coefficient for the respiratory exchange of the grasshopper should be so much lower than that found for other forms is difficult to explain. Two plausible explanations suggest themselves, however. First, grasshoppers may possess some nervous regulatory mechanism by which their respiratory exchange is controlled and, secondly, the imperfect control over the animals during the experiments might account for such results. No such nervous mechanism is known to exist in insects, and if these results were due entirely to imperfect control over the animals.

we should at least expect to find a much greater temperature coefficient for the higher temperatures, since the animals would then be most active. Much further investigation is necessary, however, before any satisfactory conclusions can be drawn.

d. Effects of starvation. Adult Melanoplus differentialis were starved, with water, for 96 to 120 hours and the rates of CO_2 output measured during this period. Some eighty-five specimens were individually studied and results given are taken from selected cases showing typical conditions. Table 6 gives the actual amounts of CO_2 given off and also the rate per gram per hour for males and females of different weights.

An examination of this table shows that the actual amount of CO_2 given off by an animal decreases during successive periods of starvation. For example, the male weighing 1.0798 grams, at the start gave off 0.000704 gram of CO_2, and at the end of 120 hours of starvation, only 0.00033 gram, a decrease of over one-half of the original amount. Since we already know that a loss in weight takes place during starvation, it is of interest to find that a decrease in the rate of CO_2 output also occurs. During the early period of starvation this decrease tends to be rather slight and gradual, but at approximately forty-eight to seventy-two hours marked drops are noted. This decided decrease is doubtless due to the fact that at this time all residual food in the intestine has been utilized and body reserves alone are being used. Figure 6 shows that males have the higher rate of CO_2 output and that these decreases are more marked for them. This is perhaps closely related to the difference in size between the animals of the two sexes. It is evident, then, from these results, that in grasshoppers as in other cold-blooded animals, frog (Hill, *25*), Planaria (Hyman, *26;* Child, *19;* Allen, *20*), CO_2 output decreases during starvation—at first rather rapidly and later reaching a practically constant level up to the time of death.

e. Effects of feeding starved animals. It is a well-known fact that in higher forms, including man, ingestion of food after starvation results in an increased rate of metabolism. Recently Lund (*27*) has found similar results for Paramecium. Various

experiments with starved grasshoppers also show striking results. Figure 6, taken from typical cases, shows the effect of feeding animals sprouted oats after periods of starvation, varying from 48 to 120 hours.

It is evident, from figure 6, that feeding increases the CO_2 output of starved animals. Some variations in extent of response

TABLE 6

Showing the actual CO_2 per one-half hour and the rate of CO_2 output per gram per hour, during starvation, for Melanoplus differentialis. Figures in italics representing CO_2 in grams per gram total body weight per hour

WEIGHT OF ANIMAL	SEX	NORMAL	STARVATION PERIOD					TEMPERATURE
			24 hours	48 hours	72 hours	96 hours	120 hours	
grams								
2.0500	♀	0.0006270	0.0004840	0.0005940	0.0006600	0.0004510	0.0004400	21
		0.0006116	*0.0005792*	*0.0006172*	*0.0006760*	*0.0004832*	*0.0004730*	
1.0100	♂	0.0006380	0.0005390	0.0007260	0.0004620	0.0005830	0.0005940	21
		0.0012360	*0.0011170*	*0.0015120*	*0.0009150*	*0.0011770*	*0.0012310*	
1.8995	♀	0.0008030	0.0007590	0.0008140	0.0006160	0.0005610	0.0007049	21
		0.0008454	*0.0008116*	*0.0009170*	*0.0007468*	*0.0007216*	*0.0007645*	
0.8995	♂	0.0007810	0.0006160	0.0005170	0.0004840	0.0003366		22
		0.0017360	*0.0015020*	*0.0012840*	*0.0012100*	*0.0008634*		
0.7795	♂	0.0006490	0.0004840	0.0004840	0.0004180	0.0002486		22
		0.0016650	*0.0014560*	*0.0013450*	*0.0012030*	*0.0007106*		
2.1200	♀	0.0010340	0.0010070	0.0009790	0.0007040	0.0005940	0.0006270	22
		0.0009754	*0.0010410*	*0.0010300*	*0.0007650*	*0.0006562*	*0.0007130*	
0.9645	♂	0.0006690	0.0006690	0.0006446	0.0004620	0.0003740		23
		0.0013680	*0.0016290*	*0.0015720*	*0.0011840*	*0.0009906*		
1.9100	♀	0.0013970	0.0010010	0.0008860	0.0006600	0.0003300	0.0003300	23
		0.0014620	*0.0013080*	*0.0011220*	*0.0009040*	*0.0006970*	*0.0006740*	
1.0798	♂	0.0007040	0.0007920	0.0007100	0.0006600	0.0005280		21
		0.0013030	*0.0014940*	*0.0013930*	*0.0013400*	*0.0006004*		
1.2148	♂	0.0006160	0.0006380	0.0008200	0.0006820	0.0005500	0.0005280	21
		0.0010140	*0.0011650*	*0.0016750*	*0.0013640*	*0.0010420*	*0.0010770*	

occur, but generally it has been found that the rate of output is approximately doubled three hours after feeding. The effects of a single feeding, however, last but a short time, depending upon the amount of food eaten. No detailed study of the effects of different amounts of different foods has been made, but an animal starved for forty-eight or more hours and then fed always shows an increased output. This increase gradually rises as the weight increases until the animal gains its normal weight relations. Such results, showing that starved grasshoppers respond to ingestion of food by increased production of CO_2, agree with those for other forms, and especially with those of Allen (*20*) for Planaria.
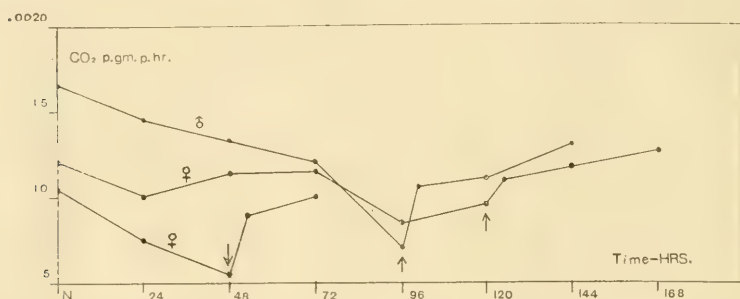
Fig. 6   Curves show decrease in CO_2 output by Melanoplus differentialis during starvation and increase after feeding. Abscissas represent time in hours indicated by numbers. Ordinates represent rate of CO_2 output in grams CO_2 per gram total body weight per hour. Time at which feeding was begun indicated by arrow. See text for further description.

CONCLUSION

The results of the present study, as presented above, seem to indicate the extent to which comparisons between some of the physiological phenomena of insects and mammals can be made. It is found that the percentage of water an animal contains is characteristic for the particular species, and that it decreases with age and increasing body weight. When exposed to low temperatures, the animals respond by a decrease in water content and are thus prevented from freezing and possible destruction.

Starvation results in marked and rapid losses in body weight, water, and solids, but the greatest and quickest loss seems to be of water. Closely correlated with these losses is a decrease in the rate of CO_2 output. Various species of animals seem to have different rates of respiratory exchange, but all show a higher rate for the younger individuals. Increased temperatures cause increased rates of CO_2 output, while lower temperatures seem to have the reverse effect. Ingestion of food by starved animals greatly increases the rate of CO_2 production. By a comparison of these data with those found for mammals, striking similarities are found to exist, and these would seem to indicate that the problem of insect physiology, although at first seemingly unrelated to that of mammals, has, in fact, many points in common with it.

SUMMARY

1. The percentage of water an animal (grasshopper) contains decreases with age and increasing body weight, up to a minimum for the species.

2. Different species of the same general group, living upon similar foods, may have different percentages of water.

3. During the active life-cycle of Chortophaga viridifasciata, the water content falls to a minimum during 'hibernation,' rises again to a maximum when 'hibernation' is broken up, and then again falls to a minimum as the animal grows old. These changes seem to be due to the effects of temperature and advancing age.

4. Water and temperature are the controlling factors in Chortophaga's emergence from 'hibernation.'

5. Different species of grasshoppers studied, under similar conditions, survive starvation for different periods of time, e.g., Melanoplus differentialis, 172 hours; Melanoplus f. rubrum, 144 hours, and Chortophaga viridifasciata, 170 hours.

6. Starvation results in losses of body weight, water, and solids, the greatest relative loss being of water. With water alone, losses are lower than with nothing.

7. Larger individuals tend to lose relatively greater amounts during starvation.

8. Rates of CO_2 output differ for the different species of animals studied.

9. Lighter and younger animals have the higher rates of CO_2 output, and the possible relation of a surface law holding for grasshoppers is indicated.

10. Temperature influences on the CO_2 production are rather marked, higher temperatures cause increased rates of CO_2 output and lower temperatures tend to have the reverse effect. However, the temperature coefficients for these different temperatures are variable and are also considerably lower than those found for other biological processes.

11. Starvation causes a decrease in the rate of CO_2 output.

12. Feeding starved individuals results in an increase in the rate of CO_2 output.

LITERATURE CITED

1 CHILD, C. 1915 Senescence and rejuvenescence. Chicago.
2 MATHEWS 1916 Physiological chemistry, 2nd ed. New York.
3 MINOT 1908 Age, growth and death. New York.
4 HATAI 1917 Am. Jour. Anat., vol. 21, p. 23.
5 LUGGER 1897 Orthoptera of Minnesota.   •
6 MORSE 1896–97 Psyche, vols. 7 and 8.
7 LUND 1918 Biological Bulletin, vol. 34, p. 105.
8 BREITENBECHER 1918 Carn. Inst. Pub., no. 263
9 BABCOCK 1912 Research Bull., Agr. Exp. Stat., Univ. Wisconsin, 19 to 24.
10 DONALDSON 1911 Jour. Morph., vol. 22, p. 663.
11 DUFOUR 1833 A l'Acad. d. Sc., T. 4, p. 129.
12 RILEY AND JOHANNSEN 1915 Handbook of Med. Ent. Ithaca, New York.
13 HATAI 1917 Carn. Inst. Publ., no. 251.
14 MORGULIS 1911 Arch. f. Entw. d. Org., Bd. 32, S. 169.
15 DONALDSON 1911 Jour. Comp. Neur., vol. 21, p. 139.
16 VERNON 1897 J. Physiol., vol. 21, p. 443.
17 BENEDICT AND EMMES 1915 J. Bio. Chem., vol. 20, p. 253.
18 RUBNER 1883 Zeit. f. Biol., Bd. 19, S. 536.
19 CHILD 1919 Am. J. Physiol., vol. 48, p. 231.
20 ALLEN 1919 Am. J. Physiol., vol. 49, p. 420.
21 KROGH 1916 The respiratory exchange of animals and man. Longmans, Green & Co., London and New York.
22 MEEH 1879 Zeit. f. Biol., Bd. 15, S. 425.
23 CLAUSEN 1890 Landwirtschaftl. Jahrb., Bd. 19, s. 893.
24 MATTHAEI 1904 Trans. Philos. Soc. London, series B, vol. 197, p. 47.
25 HILL 1911 J. Physiol., vol. 43, p. 379.
26 HYMAN 1919 Am. J. Physiol., vol. 49, p. 377.
27 LUND 1918 Am. J. Physiol., vol. 47, p. 318.

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Espermatogénesis de la mosca Asilus sericeus Say.

Asilus sericeus Say es una especie de Díptero sumamente favorable para los estudios sobre la espermatogénesis a causa del considerable número de células y de la exacta serie de estados de crecimiento en el testículo, así como la ausencia relativa de estados confusos durante el periodo de crecimiento. Existen cinco pares de cromosomas. La asociación de los cromosomas en parejas se presenta en la espermatogonia y se retiene durante la última división espermatogonial. En la telofase de esta división, la disposición de los cromosomas en parejas es tan íntima que realmente equivale a la sinapsis, y la unión que tiene lugar en este momento persiste durante el periodo de crecimiento que la sigue.

Los autores no han podido encontrar estados leptoténicos o zigoténicos (sinápticos) propiamente dichos. Los cinco elementos dobles (bivalentes) que aparecen en la telofase permanecen relativamente condensados y son fácilmente discernibles durante todo el periodo de crecimiento hasta la primera división de los espermatocitos. La sinapsis precoz y la omisión del estado leptoténico ordinario pueden interpretarse como una manifestación de la fuerza que origina la asociación por parejas característica de los cromosomas de la espermatogonia, oogonia y células somáticas de los dípteros.

SPERMATOGENESIS IN THE FLY, ASILUS SERICEUS SAY[1]

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Station for Experimental Evolution, Carnegie Institution of Washington

TWO PLATES (TWENTY-TWO FIGURES)

CONTENTS

INTRODUCTION

The rapid development of genetical studies on Drosophila in the last few years has drawn considerable interest to the subject of gametogenesis in the Diptera. The phenomena of mutation, crossing over, nondisjunction, etc., occur partly or wholly during gametogenesis, making it desirable that our knowledge of the latter be extended as far as possible. Another feature that has added interest to the subject is the characteristically 'paired association' of the somatic chromosomes of flies as distinguished from those of other animals.[2] It seemed possible that this pre-existent association of chromosomes in the soma and early germ cells might affect the processes of maturation in such a manner as to throw additional light on their significance.

[1] We are indebted to Mr. C. W. Johnson for identifying the species used.
[2] For discussion of this phenomenon, see Metz, '16.

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These considerations led the senior author some time ago to begin gathering material for a study of gametogenesis, in connection with other studies on Diptera chromosomes, and the first results of this study are presented here. From the genetical standpoint the present paper represents only a short step in the desired direction, but it may serve as a foundation upon which to base further work—particularly studies on oögenesis, which are under way at the present time.

Two reviews of the literature on Diptera chromosomes have been given recently (Metz, '16, pp. 213, 214; Whiting, '17). From these it will be seen that most of the work on the subject has dealt primarily with the sex chromosomes or other special features, and that our knowledge of gametogenesis, especially the growth stages, is meager.

In her studies on the sex chromosomes, Stevens ('07, '08, '10, '11) records several observations on other aspects of spermatogenesis, but unfortunately these cannot be combined to make a connected account. The observations of Taylor ('14) and of Lomen ('14) on Culex, in addition to being meager as regards details of spermatogenesis, are, we believe (see also Whiting, '17), faulty on account of the poorly fixed material used. Whiting ('17), in a more recent paper on Culex, has given a comprehensive account of the maturation divisions, beginning with the first spermatocyte prophase. His observations on the earlier stages, however, particularly the earlier part of the growth period, are limited, probably owing to the fact that Culex does not afford favorable material for this purpose.

As regards oögenesis in the Diptera, practically nothing has been published, so far as we are aware.

Unfortunately, the Diptera have long, and justly, been looked upon as unfavorable objects for cytological study—a fact that has undoubtedly been responsible for keeping our knowledge of gametogenesis in this group far behind that of such insects as the Orthoptera, Hemiptera, and Coleoptera. We have found, however, that some groups of Diptera are much more amenable to study than others, and by making selections from these and by careful attention to technique we have been able to obtain

favorable material. It is hoped that the results obtained from this will eventually permit of a satisfactory analysis of the processes in the difficult Drosophila material.

Our survey of the Diptera has not yet revealed any single species that is favorable for a study of all stages of spermatogenesis, but the species considered here combines more favorable features than any other. In this form the seriation of stages through the development of the first spermatocyte up to the maturation divisions is clear-cut and practically complete. Details of certain stages are not depicted with as great clearness as they are in some other species (to be considered subsequently), but in most respects the material is unusually favorable.

TECHNIQUE

Flemming's strong solution has been found most satisfactory for fixation and has been used almost exclusively. Heidenhain's iron haematoxylin and safranin have been used for chromatic stains, with or without counter-stains. A more detailed consideration of technique has been given in an earlier paper (Metz, '16, p. 219). All of the accompanying figures are from material fixed in Flemming and stained in iron haematoxylin.

TOPOGRAPHICAL FEATURES

The testes in Asilus, like those in most other asilids, are a pair of long coiled tubes, each containing thousands of cells. The distal end of the testis contains a clearly marked spermatogonial region with a central core of giant nutritive cells. Then comes a narrow transition zone in which spermatogonial anaphases and telophases are intermingled with the earliest stages of the first spermatocytes. The nuclei here are very small. Following this is a broad zone, containing cells, nearly all of which are in one stage. From this point the development involves a gradual transformation, which may be followed with comparative ease through the long growth period extending far down the testis. In favorable material all of these stages appear in one preparation, or even in one section, and since there is such

a large number of cells available for study, even the intermediate transition stages, often so difficult to obtain, are nearly all in serial order. The only exceptions to this rule are found in the stages immediately following the last spermatogonial anaphases. Here development proceeds with great rapidity, and the telophase and associated stages are more or less intermingled. But in spite of this it is possible to obtain a clear conception of what takes place, for the immediately succeeding stage (b) is perfectly clear, and with its aid the other figures may be pieced together. The details of these processes will be given below.

SPERMATOGONIA

The spermatogonia are abundantly represented in our material and are of ample size for study. Those of the last generation or two are noticeably smaller than the earlier ones and have the chromosomes more closely aggregated in metaphase, but otherwise are not appreciably different from the rest. In a previous paper (Metz, '16) the general peculiarities of chromosome behavior in spermatogonia and other diploid cells of flies have been described in detail. For this reason the spermatogonial stages will be passed over briefly here. It is important, however, to keep in mind the fact that the close association of homologous chromosomes, especially in the resting stages and prophases, makes the pairs of chromosomes simulate single chromosomes of other animals.

In spermatogonial metaphases of A. sericeus there are ten chromosomes, arranged in five pairs (figs. 3 and 4), the smallest of which is probably the sex chromosome pair, although there is no evident dimorphism to distinguish it. Occasionally the arrangement of one or two pairs is disturbed, but normally they all show the paired association just as in other Diptera (Metz, '16). In anaphase the chromosomes pass to the poles in this paired condition (fig. 5). Their behavior during the telophase will be discussed below.

During most of the resting stage the chromatin is diffuse and stains so faintly that its behavior cannot be observed satisfactorily. In prophase it becomes aggregated, and each aggre-

gate gives rise to a long double thread by a process of attenuation or uncoiling (fig. 1). This thread shortens up immediately into a pair of prophase chromosomes (fig. 2).[3]

FINAL SPERMATOGONIAL TELOPHASES

Since the telophase of the last spermatogonial division is a stage of particular interest, it may be considered separately. In the last spermatogonial anaphase, as in preceding anaphases, the chromosomes go to the poles associated in pairs. In late anaphase the pairing becomes more intimate, due partly to the crowding of the chromosomes at the poles. Then in telophase the crowding is relaxed, the cluster loosens up, and the individual chromosomes may be observed. They are now intimately associated in pairs—so intimately, indeed, that the duality is often obscured. In other words, as the cluster loosens, the chromosomes separate out as bivalents instead of single elements. In figures 6 and 7 different degrees of association are represented. Some of the chromosomes show the dual structure clearly, while others show it very little or not at all. These nuclei are entire, or nearly so, and all of the chromatin is represented. Figure 8 is from a slightly later stage in which the paired association is so intimate that all trace of duality is gone, and only five chromosomes can be detected.

The staining capacity of the chromatin is greatly decreased at this time and the chromosomes appear less bulky than before. Needless to say, such figures are difficult to analyze, but a careful study has convinced us that the process is as described—that homologous members join in early telophase and effect an intimate union side by side. This conclusion is based not only on the duality of the telophase chromosomes, but on the fact that they are haploid in number (5) instead of diploid (10). The cells are small, affording plenty of examples of uncut nuclei, and in no case have we been able to find one in which the chromatic bodies approached the diploid number. Indeed, we found no clear case in which more than five were present.

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[3] These features, together with other details omitted in the present paper, will be considered more fully in a subsequent publication.

Probably the density of the stain or degree of extraction has a good deal to do with the appearance or non-appearance of the duality in these telophase nuclei, but there can be no doubt that the union is very intimate. In this relation the chromosomes pass (fig. 9) into the succeeding stage in which they lose their staining capacity to a much greater extent, as they enter the growth period. A careful scrutiny of the late telophase nuclei reveals very little indication of a spinning-out process or a network formation, except that due to the linin. The chromosomes appear simply to fade out through loss of color, while retaining, approximately, their form and position (fig. 9).

It is probable that the above account should not be restricted to the final spermatogonia, but should apply to all of the spermatogonial telophases. The evidence points consistently in that direction (Metz, '16), but we have not been able to make sure of the point in the species under consideration.

THE EARLY GROWTH PERIOD; STAGES A AND B

Following the final spermatogonial telophase there is a very brief period during which relatively little chromatin is visible in the nucleus, as indicated by figure 10. This stage, which may be called stage a, is also characterized by the appearance of a small nucleolus, as shown by the figures. The nuclei of this period, together with those of the telophase just preceding, are the smallest to be found in the testis and cannot be confused with those of any other stage.

Apparently our stage a corresponds to Montgomery's stage a in the Orthoptera and Wilson's stage a in the Hemiptera (see below). So far as we can determine, it is structurally similar to the early resting stage of the spermatogonia. The stage is so brief that it is only represented by a few scattered groups of cells at the border between the final spermatogonia and the clearly marked region in which the next stage (stage b) is represented.

Adjacent to the nuclei of stage a (fig. 10) are others only slightly larger in which the chromatin becomes progressively more deeply stained and condensed, revealing the outline of the chromosomes.

These are double and haploid in number, corresponding to the telophase pairs. The size of the cells and nuclei indicate that the actual growth period has barely begun when these bodies become visible (fig. 11), and there seems to be little doubt that the preceding transition from the telophase stage has not only been very brief, but has involved little change in the chromosomes other than that involved in the loss of staining capacity. The intimate association in pairs appears to have remained unaltered.

The chromatin now becomes further condensed, revealing the size and shape of the bivalents in a more clear-cut manner (figs. 12 and 13). Attached to one of these (apparently the smallest pair) is the nucleolus, the history of which will be considered later. This stage may be designated stage b. Structurally stage b resembles the late resting stage or early prophase of the spermatogonia in which the chromatin becomes condensed into five bivalent aggregates that give rise to the prophase chromosomes.[4]

Since stage b forms a definite point of orientation between the brief early stages and the more extended later ones, it may be well to consider events up to this time before describing the subsequent processes. It is apparent that the synaptic condition has been fulfilled at the very beginning of the growth period by the intimate association in telophase of chromosomes that were, for the most part,[5] already arranged in pairs. Technically, this association should be called synapsis, for, as will be seen, the union effected in telophase persists throughout the growth period and is responsible for the formation of the bivalent chromosomes of the first maturation division.

Compared with the corresponding stages in other animals, this behavior seems to be unique, and it seems legitimate to infer that it is associated in some causal manner with the other

[4] See footnote 3, page 169.

[5] It is not justifiable to assume that the paired association in anaphase is absolute and invariable, for occasionally the two members of a pair may be separated in metaphase (e.g., fig. 4), and consequently in anaphase. It must be assumed, however, that in these exceptional cases the paired arrangement is restored in telophase or soon thereafter.

peculiarity of the dipteran chromosomes—their characteristically paired association in somatic cells.

It is of interest to consider the events up to this point in relation to those found in other insects, such, for instance, as the Hemiptera, Odonata, and Orthoptera. A marked similarity is at once noticeable in many features, but always with the difference that the chromatic elements of Asilus are double instead of single.

Thus in Oncopeltus Wilson ('12) describes the stages immediately following the final spermatogonial divisions as involving a diffusion of the chromatin in telophase followed by a stage in which definite prochromosome-like aggregates arise (compare Wilson's figures 48 to 51 with our figures 9 to 13.)[6] These aggregates or masses would correspond to those of stage b in Asilus, but instead of being haploid in number and bivalent in composition, they are, in Oncopeltus, diploid in number and apparently univalent in composition.

In Lygaeus among the Hemiptera (Wilson, '12), Anax among the Odonata (Wilson, '12), Phrynotettix, Dissosteira, and Chortophaga among the Orthoptera (Davis, '08; McClung, '02; Wilson, '12), and probably in numerous other forms, phenomena not essentially different from those in Oncopeltus are found, so that the comparison of Asilus with Oncopeltus may be extended to include several species representing a widespread type of spermatogenesis as regards the earliest stages of the growth period.

Apparently the Coleoptera may also be put in this class, although there are so many conflicting accounts of coleopteran spermatogenesis that many cases are open to question. The essential features, however, namely, the resting stage followed by the appearance of more or less condensed masses or aggregates in diploid number, seem to be well established in certain instances (Stevens, '05, '06, '08 a, '09; Nonidez, '14, '15; Goldsmith, '19, figs. 17, 18, 19).

[6] This resemblance is even more strikingly shown by another species of Asilus (A. notatus) in which the prochromosome-like bivalents are more condensed and shorter than in A. sericeus (discussion, page 178).

One author (Arnold, '08) has described in Hydrophilus piceus (Coleoptera) a precocious reduction of the chromosomes at the beginning of the growth period not unlike that found in Asilus. But the brevity of his description together with the fact that no other observer (Stevens, Vom Rath, '92, Goldsmith, etc.) has noted such a phenomenon in this or other Coleoptera makes it seem probable that Arnold is mistaken in his interpretation.

It appears, then, that although a superficial similarity exists between the early growth stages of Asilus and those of various other animals, the divergence between the double (bivalent) chromatic bodies on the one hand and the single ones on the other separates the representative of the Diptera from all the other forms.[7]

If we turn to the plants, however, we find a different situation. Here, although the evidence is not as clear as might be desired, some species appear to exhibit a paired association of 'prochromosomes,' in the early growth period immediately after the last diploid telophase, somewhat like that found in Asilus. Overton ('05, '09), for instance, records such an association in Thalictrum, Calycanthus, Campanula, and Helleborus. In these the last diploid division is followed by a resting-stage network in which definite chromatic bodies (prochromosomes) are scattered about. These are diploid in number, but often, or usually, lie in paired association. Their shape and degree of condensation differ in different cases, but their paired association seems to be fairly constant. In these plants the association may persist from the last 'premiotic' anaphase through the growth period and up to the metaphase of the reduction division, although Overton does not commit himself as to the behavior in the telophase and earliest stage of the growth period, as indicated by the following:

[7] The earlier literature of spermatogenesis contains numerous references to possible or supposed precocious pairing in the last spermatogonial telophases. For instance, Montgomery, '00, page 297, on Peripatus, notes a few such 'exceptional' cases; Blackman, '03, '10, page 141, on Scolopendra, describes a precocious telosynapsis; Stevens, '03, on Sagitta, suspects an early pairing, and Downing, '05, on Hydra, makes a similar suggestion. Other and more recent examples could be cited also, but in no case have we been able to find clear-cut evidence of such an association as is exhibited by Asilus.

I have attempted to trace the processes of reconstruction of the nucleus of the pollen mother-cells from the last pre-meiotic division, and to compare the structure of these nuclei with that of ordinary somatic ones, but have experienced considerable difficulty in identifying with certainty the last pre-meiotic divisions. After the formation of the nuclear membrane and during the period of nuclear enlargement, the chromatic material becomes rather regularly distributed in the nuclear cavity, the greater portion of the stainable substances lying in the prochromosomes, each suggesting by its form and size that it is derived from a chromosome of the preceding telophases. I am not prepared to discuss the problem as to how the chromosomes of the telophases are modified in passing over into the resting nucleus. (Overton, '09, pp., 21, 22.)

In Oncopeltus and the other insects mentioned above, the prochromosome-like bodies of stage *b*, whether massive (Wilson's stage *b* in Hemiptera and Anax) or more thread-like (Davis's stage *b* in Orthoptera), give rise, by a process of unraveling, to long, delicate leptotene threads that then undergo synapsis to form the pachytene or diplotene threads. Since, in Asilus, the chromosomes are already double (i.e., bivalent) it is of especial interest to examine their subsequent behavior.

LATER GROWTH PERIOD

The transition from stage *b* to later stages involves merely a gradual lengthening out of the five diplotene threads (figs. 13 to 16) and their polarization with reference to the nucleolus. One member (apparently the smallest) is already attached to the nucleolus. The others, or at least two or three of them, soon become attached and extend out like fingers (figs. 16 to 20). Apparently each thread becomes attached at one end only. No cases have been found in which a complete loop was formed. Fortunately, the threads lie close to the nuclear wall and remain well separated from one another throughout almost the entire growth period, so they may be examined readily. They show no indication of dissociation into single (leptotene) threads at any stage, although their duality is evident throughout. As may be noted from the figures, the nucleus decreases somewhat in size instead of enlarging as polarization progresses.

The polarized stage persists almost up to the first spermatocyte prophase, and is modified, toward the end, by a definite contraction period (fig. 17) in which the threads draw away from the nuclear wall and lie close together. Apparently no significance attaches to this contraction for the threads undergo no visible changes and soon spread out again into their previous positions near the periphery (figs. 20 and 21) and condense into the five prophase chromosomes, ready to go on the spindle.

Although these processes cover about four-fifths or more of the growth period and are represented by many thousands of cells, they are so simple and involve such slight changes in the chromatin, that in essentials the condition found in stage b (fig. 13) may be said to typify all the succeeding stages up to the prophase, and the whole series may be represented by a few figures. The diplotene threads that appeared at the beginning of stage b have persisted unchanged so far as their diplotene structure is concerned. The contraction stage, occurring in the late growth period, if it has any counterpart, outside of the Diptera, would represent the so-called second contraction, taking place long after synapsis.

These events seem to resemble those in Thalictrum (Overton, '09) to the extent that the chromatin remains in the form of relatively condensed, bivalent threads. Compared with animals, however (other than Diptera), there is no such resemblance, for, as just mentioned, the leptotene and synaptic stages usually following stage b are not found in Asilus.

THE SPERMATOCYTE DIVISIONS

Since this paper is concerned primarily with the growth stages, the maturation divisions will be passed over briefly. So far as known, they present no unusual features. Metaphases of both spermatocyte divisions are clear, and each shows five chromosomes. Only the first is represented here (fig. 22). It is the reduction division, apparently, for no tetrad structure is evident.

THE NUCLEOLUS

The history of the nucleolar structures has not been studied in detail but the nucleolus is so prominent during the growth period that a study of the chromosomes must necessarily reveal the main features of the nucleolar behavior. It is probable that the chromatic part of the nucleolar complex persists from the final spermatogonial anaphase in the form of a pair of chromosomes, but whether the achromatic portion arises from this or originates independently we are unable to state. The two are united from stage *b* (fig. 11) throughout the remainder of the growth period. The chromatic portion may be followed directly to the first spermatocyte metaphase where it becomes one of the five bivalent chromosomes. During much of the growth period the nucleolus is plainly compound (fig. 14), being composed of a large, oval achromatic portion and a smaller dense chromatic portion to which is attached a chromatic thread or finger-like projection. The latter is very characteristic and persistent throughout the growth period. The achromatic portion seems to diminish gradually during the later stages, and cannot be detected with certainty in late prophase. However, the degree of extraction of the haematoxylin has much to do with the appearance of the structure, and it is difficult to say just what becomes of the achromatic portion.

The chromatic portion is presumably the sex chromosome pair. At first sight the finger-like process suggests the presence of an unequal XY pair, but this asymmetry seems to disappear in metaphase and we are unable to verify the point. Likewise, the spermatogonial divisions do not reveal any such inequality in any chromosome pair. It seems more probable that the finger-like process is due to a difference in the degree of condensation of the two chromosomes, such, e.g., as that shown by the XY chromosomes of Enchenopa binotata (Kornhauser, '14).

In another species of Asilus it is practically certain that the chromosomes involved in the nucleolar complex are the sex chromosomes, and it may be inferred that the same is true in A. sericeus. This is in agreement with the observations of Stevens ('08 b), who found the sex chromosomes condensed during the growth period in several species of flies.

DISCUSSION

Asilus sericeus presents the most simple and clear-cut type of spermatogenesis thus far found in the Diptera, owing to the fact that during the growth period the chromatic threads do not spin out and become entangled to such a degree as they do in other forms.

When compared with animals other than Diptera, the most outstanding characteristic of the maturation processes in Asilus is the apparently continuous association of corresponding chromosomes in pairs. Superficially some of the stages bear a marked resemblance to those in various other forms, but on close examination it appears that only the later growth period and succeeding stages are actually similar in essential features. Previous to this there is an underlying difference due to the fact that in Asilus the chromosomes, whether condensed or thread-like, maintain an intimately paired association from the telophase throughout the entire growth period, with the result that the usual leptotene stage and the subsequent synaptic process seem to be omitted entirely. This is discussed more specifically above.

Another feature that should be recalled here is the probable parallelism between the peculiarities in chromosome behavior observed in Asilus and those found in certain plants as recorded by Strasburger, Rosenberg, Müller, Overton, and others (see especially Overton, '09). Apparently the peculiarities during the maturation stages are in each known case correlated with a noticeable paired association of chromosomes in the somatic cells, which would again lead one to conclude that the two phenomena are causally connected and are both manifestations of the same inherent 'tendency toward pairing.' As has been remarked previously (Metz, '16, p. 225), the latter seems to be an accentuation of the tendency or force that unites corresponding chromosomes during synapsis in most organisms. It seems to differ mainly in that its effects in the cases mentioned are not limited to the final germ cells, but are visible in somatic and early germ cells as well. What this force is, physicochemically, remains as obscure as ever, although there is very strong reason for believ-

ing that it is due to a *likeness in constitution* of corresponding chromosomes.

Regarding the genetical question of 'crossing over,' our observations afford only negative evidence. Since no leptotene threads have been observed and nothing like a typical synaptic stage has been identified, there is little indication of any process that might bring about crossing over during the early stages. It is difficult to determine just what takes place in stage *a*, but it should be recalled that this stage appears to be like the spermatogonial resting stage, and that as far as cytological evidence goes there is no more reason for expecting it in the former than in the latter.

In subsequent stages there is some evidence of chromosome twisting, but more often the threads lie side by side without twisting, and when they do overlap there is no evidence of breaking. On the whole, then, what evidence there is would argue against the probability of crossing over in the males of Asilus, which agrees with the genetical results in Drosophila, where crossing over is found only in the female. But this question, from the cytological standpoint, is only in the speculative stage, and will probably remain there at least until further studies are completed, particularly studies on oögenesis.

In this connection a word should be said regarding the degree to which the above description may be considered typical of the Diptera. One other species of Asilus (A. notatus) has been studied fully and shows certain noteworthy deviations from the above account. These may be summarized briefly as follows: In stage *a* following the final spermatogonial telophase, the chromatin stains more deeply than in A. sericeus and gives even clearer evidence of remaining relatively condensed, i.e., not spinning out into threads. Stage *a* is very brief and is succeeded immediately by stage *b*, in which the chromatin is likewise more dense than that in the corresponding stage of A. sericeus. It is in the form of short, thick, bivalent prochromosome-like bodies, the dual nature of which is very plain. These show a more marked superficial resemblance to the bodies of stage *b* in the Hemiptera than do those of A. sericeus.

The most noticeable difference between sericeus and notatus, however, appears in the stage immediately following stage *b*. At this time the bivalents in A. notatus, instead of lengthening only slightly and remaining well separated from one another, as they do in sericeus, become greatly attenuated and entangled for a time, making analysis very difficult. Here again the superficial resemblance to phenomena in the Hemiptera is more marked than in sericeus, although the actual structural characteristics (persistence of the diplotene condition) appear to agree with those of sericeus.

When an attempt is made to compare spermatogenesis in Asilus with that in other genera of flies, confusion enters at once. Other members of the Asilidae show definite resemblances, but outside of the family superficial differences are so great that comparisons cannot be made safely without very careful study. As a case in point we may mention the genus Drosophila. Superficially spermatogenesis in this group is exceedingly different in appearance from that in Asilus. Further study and possibly detailed examination of intermediate forms will be necessary before the relationships can be determined. Perhaps much of the apparent divergence between Drosophila and Asilus is due to difference in the cytoplasm, rate of growth of the spermatocytes, degree of staining of the different nuclear elements, and other secondary features, but there is as yet no certainty that it may not also include fundamental differences in the chromosomal behavior.

SUMMARY

1. The spermatogonial chromosomes of A. sericeus are ten in number, arranged in five pairs. The sex chromosomes have not been identified.

2. In the last spermatogonial anaphase, as in preceding anaphases, the chromosomes go to the poles associated in pairs.

3. The paired association becomes more intimate in telophase, giving rise to bivalent chromosomes in haploid number.

4. A brief diffuse stage (stage *a*) ensues, in which the chromatin stains only slightly.

5. Then the double chromosomes reappear, apparently in the same form and relative position as before, and condense into bivalent prochromosome-like bodies (stage *b*).

6. The ordinary leptotene condition seems to be omitted entirely.

7. The bivalent bodies of stage *b* elongate into diplotene threads that remain relatively condensed and clearly separate throughout the entire growth period, giving rise to the bivalent prophase chromosomes.

8. In another species, A. notatus, the process appears to be essentially the same, but is somewhat confused by a spinning out and intertwining of the threads in the stage following stage *b*.

9. The usual synaptic process is entirely wanting. Synapsis is effected in telophase at the beginning of the growth period by an intimate association of chromosomes that were already paired in anaphase.

10. Superficially the early growth stages are not unlike those in the Hemiptera and other forms, but the chromatic structures are bivalent instead of univalent.

11. Tetrad structures are not visible.

12. The first division appears to be reductional for all of the chromosomes.

LITERATURE CITED

ARNOLD, G. A.   1908   The nucleolus and microchromosomes in the spermato-
        genesis of Hydrophilus piceus.   Arch. f. Zellfor., Bd. 2, No. 1.
BLACKMAN, M. W.   1903   The spermatogenesis of the myriapods II.   Biol. Bull.,
        vol. 4; pp. 187–218.
        1910   The spermatogenesis of the myriapods VI.   Biol. Bull., vol. 19;
        pp. 138–160.
DAVIS, H. S.   1908   Spermatogenesis in Acrididae and Locustidae.   Bull. Mus.
        Comp. Zool. Harvard, vol. 52, no. 2.
DOWNING, E. R.   1905   The spermatogenesis of Hydra.   Zool. Jahrb., Bd. 21;
        s. 379.
GOLDSMITH, W. M.   1919   A comparative study of the chromosomes of the tiger
        beetles.   Jour. Morph, vol. 32, pp. 437–488.
KORNHAUSER, S. I.   1914   A comparative study of the chromosomes in the
        spermatogenesis of Enchenopa binotata, etc.   Arch. f. Zellf., Bd. 12,
        s. 241–298.
LOMEN, FRANZ   1914   Der Hoden von Culex pipiens L.   Zeits. Naturwiss., B. 52.

McClung, C. E. 1902 The spermatocyte divisions of the Locustidae. Kans. Univ. Sci. Bull., vol. 1, pp. 185–240.

Metz, C. W. 1916 Chromosome studies on the Diptera II. Jour. Exp. Zoöl., vol. 21, pp. 213–279.

Montgomery, T. H. 1900 The spermatogenesis of Peripatus. . . . . Zool. Jahrb., Bd. 14, s. 277–368.

Nonidez, José 1914 Los cromosomas en la espermatogenesis del 'Blaps lusitanica' Herbst. Trab. Mus. N. Cienc. Nat., Madrid, Ser. Zool., num. 18.
1915 Estudios sobre las celulas sexuales. I. Los cromosomas goniales y las mitosis de maduracion en Blaps lusitanica y B. Waltli. Mem. Soc. Esp. Hist. Nat., T. 10.

Overton, J. B. 1905 Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen. Jahrb. f. wiss. Bot., Bd. 42, s. 121–153.
1909 On the organization of the nuclei in the pollen mother cells of certain plants. Ann. Bot., vol. 23, pp. 19–62.

Stevens, N. M. 1903 On the oögenesis and spermatogenesis of Sagitta bipunctata. Zool. Jahrb., Bd., 18, s. 227–240.
1905, 1906 Studies in spermatogenesis. Parts I and II. Carnegie Institution of Washington, Pub. 36.
1907 The chromosomes of Drosophila ampelophila. Proc. VII Internat. Zool. Cong.
1908 a The chromosomes in Diabrotica. . . . . Jour. Exp. Zoöl., vol. 5.
1908 b A study of the germ cells of certain Diptera, with reference to the heterochromosomes and the phenomena of synapsis. Jour. Exp. Zoöl., vol. 5, p. 459. 359
1909 Further studies on the chromosomes of the Coleoptera. Jour. Exp. Zoöl., vol. 6.
1910 The chromosomes in the germ cells of Culex. Ibid., vol. 8.
1911 Further studies on the heterochromosomes in mosquitoes. Biol. Bull., vol. 20, pp. 109–120.

Taylor, Monica 1914 The chromosome complex of Culex pipiens. Quart. Jour. Mic. Sci., vol. 60.

Vom Rath 1892 Zur Kenntnis der Spermatogenese von Gryllotalpa vulgaris Latr. Arch. Mikr. Anat., Bd. 40; s. 102–132.

Whiting, P. W. 1917 The chromosomes of the common house-mosquito, Culex pipiens L. Jour. Morph., vol. 28, pp. 523–577.

Wilson, E. B. 1912 Studies on chromosomes VIII. Jour. Exp. Zoöl., vol. 13, pp. 345–449.

EXPLANATION OF PLATES

All of the figures were drawn from material fixed in Flemming and stained in iron haematoxylin; sections in most cases were 5μ in thickness. All were drawn with the aid of a camera lucida, using 1.5-mm. Zeiss apochromatic objective and no. 12 ocular, with 160-mm. tube length. Drawings were made at table level and are reproduced without reduction in size.

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 13, Asilus sericeus. Figures 1 to 5, spermatogonia. Figures 6 to 13, telophases of final spermatogonia and early growth stages of first spermatocyte.

1 Late resting stage or early prophase, the chromatin aggregated into five bodies that represent the five pairs of chromosomes.

2 Two prophases, nuclei entire, each showing the five pairs of chromosomes, resembling bivalents. Note the differences in degree of condensation of the different elements.

3 and 4 Early and late metaphases.

5 Final spermatogonial anaphase, showing the paired association of the chromosomes as they go to the poles; nucleus is cut so that one pair is missing from the lower pole.

6 Late anaphase or early telophase of the final spermatogonial division. The nuclei are practically entire and the chromosomes are all represented. The figure at the right is slightly earlier than the one at the left, but the union of the chromosomes in pairs is so intimate that only one shows the dual structure. In the figure at the left the duality is scarcely visible, and not more than the haploid number of chromosomes can be detected; the two lying side by side at the lower pole are separate pairs, not members of one pair.

7 Approximately the same stage as 6. The union is progressively more intimate from the lower to the higher of the three nuclei. The nuclei are practically entire and each shows four or five bivalent chromosomes.

8 An entire nucleus of the same stage, showing the five bivalent chromosomes; scarcely any trace of duality is revealed.

9 Four cells in late telophase after the nuclear membrane has appeared and the chromosomes have moved apart and lost much of their staining capacity. Only part of the chromatin is represented.

10 Stage a, slightly later than the preceding; the chromosome remains are barely visible; nuclei not entire.

11 to 13 Stage b, the chromosomes again taking the stain and reappearing in the form of five long bivalents, one of which forms part of the large nucleolus; nuclei entire.

Figures 14–22, Asilus sericeus, middle and late growth stages.

14   Late stage *b*, four of the bivalents drawing out into diplotene threads, the other attached to the plasmosome, forming the nucleolar complex.   Three of the threads are, respectively, at the top, the extreme left, and the extreme right of the figure; the fourth is at a low focus, indicated by its light color, and passes underneath the nucleolus.   The nucleus is entire.

15 and 16   Stages progressively later than the preceding, showing the condensation of the threads and their orientation with respect to the nucleolus; nuclei entire.

17   Contraction stage, nucleus entire.

18 to 20   Successive stages following the contraction.   The diplotene threads extend out like fingers from the nucleolus; nuclei entire.

21   Prophase of the first spermatocyte division; nucleus entire.   The four long threads have broken loose from the nucleolus; the latter has become smaller and is mostly chromatic.

22   First spermatocyte metaphase showing the five bivalents.
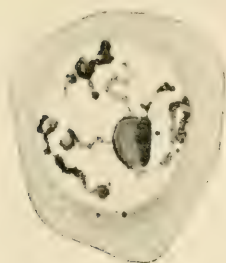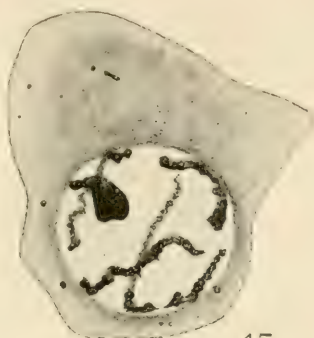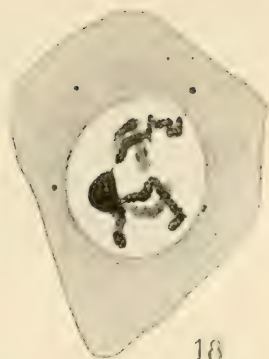
Resumen por el autor, Harold H. Plough,
         Amherst College.

Nuevos estudios sobre el efecto de la temperatura en el crossing over.[1]

Los datos presentados en este trabajo constituyen un suplemento a un trabajo precedente del autor sobre el efecto de la temperatura sobre el crossing over del segundo cromosoma de Drosophila melanogaster. Empleando el mismo método del trabajo mencionado, el autor ha estudiado los efectos de dicho agente sobre prácticamente el total de la longitud conocida de los cromosomas segundo y tercero, sometidos a una temperatura de 31.5°C. Los resultados obtenidos indican que ni la temperatura ni la edad de la hembra madre de una generación, producen una variación significativa en el crossing over de parte alguna del cromosoma sexual. Solamente la región media del tercer cromosoma presenta un aumento marcado en el crossing over como resultado de la exposición a una temperatura elevada y una variación con la edad. Las regiones de los cromosomas que son "sensitivas" a los cambios del medio ambiente presentan también una proporción elevada de crossing over sencillo y doble. Es probable que donde el crossing over es menos libre, se pueden observar los efectos del medio ambiente.

[1] Con este nombre se designa el entrecruzamiento de los cromosomas en las células sexuales de la hembra. Nos parece más conveniente conservar la palabra inglesa, puesto que ha adquirido un significado preciso que se pierde al traducirla. (N. del T.)

FURTHER STUDIES ON THE EFFECT OF TEMPERATURE ON CROSSING OVER

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THREE FIGURES

CONTENTS

INTRODUCTORY

In an earlier study of the effect of temperature on crossing over in Drosophila melanogaster I showed that temperature both above and below the optimum (22°C.) caused a significant increase in the amount of crossing over between certain genes located in the second chromosome (Plough, '17). Preliminary work on the first and third chromosomes indicated, however, that crossing over in these groups was not visibly affected by temperature. No reason for this unexpected result could be assigned, and it seemed worth while to test the first and third chromosomes by the same accurate methods which had been used with chromosome II. Such data would also give an accurate basis for checking the fact reported by Bridges ('15) that in chromosome I, unlike the second chromosome, there is no significant variation in crossing over due to the age of the female parent.

The large amount of breeding work with Drosophila has resulted—especially through the work of Bridges—in making available a large number of easily workable mutant characters

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with excellent viability. The most valuable mutants of each chromosome have been assembled into multiple stocks, the use of which has made it possible to determine the effect of environmental changes on linkage relations over approximately the whole known lengths of each of these chromosomes in a single experiment. At the same time in these multiples the distances between the different genes are generally not sufficiently great to cause complications due to unobserved double crossing over. My present more accurate data establish the truth of the earlier observation that crossing over in chromosome I is not influenced by temperature, but show that there is a section of chromosome III in which crossing over is increased in the same way as in chromosome II.

EXPERIMENTAL

The mutant stocks used for the tests were the sex (or first) chromosome multiple stock, scute-echinus-cut-vermilion-garnet-forked, and the third chromosome multiple stock, sepia-spineless-sooty-rough. Scute shows an absence of scutellar bristles, and echinus, a roughened condition of the facets of the eyes. The other mutations have been described. Dichete, a dominant character, was introduced in a small number of preliminary third chromosome tests based on ten-day brood counts. A glance at the chromosome maps in figure 3 will show that the genes used cover both chromosomes at fairly even intervals throughout a large portion of their known lengths.

The method of making the tests was essentially the same as that used in my earlier work with the second chromosome. Virgin sister females of the normal wild stock were mated to males of the multiple mutant stock to be tested, and allowed to lay in one set of bottles for about three days. This first set of bottles was kept at the control temperature. The P_1 pairs were next transferred to another set of bottles which was kept continuously at the high temperature. Virgin female offspring from each set were then isolated and back crossed to males of the original mutant stock used. These back crossed pairs were placed in quarter-pint milk bottles containing banana agar, and

kept at the control temperature. With the exception of the preliminary ten-day brood test, the pairs were changed from one set of such bottles to another at the end of successive three-day periods throughout the life of the females. (The first change was made in all cases at the end of the fourth day.) The counts of the successive sets of offspring of these pairs furnished the data for determining the effect of the high temperature on crossing over in the developing eggs of the heterozygous females.

The control temperature was approximately 24°C. maintained in a wooden stock cabinet controlled by an electric heater with a thermostat. It varied between 22°C. and 25°C. throughout the experiments. The high temperature was 31.5°C. maintained in a Freas electric incubator. This varied as much as 1° above and below, though the normal variation was about 0.5° either way.

The results given by the tests of the first chromosome regions are given in table 1. The bottle counts for each three-day period are added and the percentages of crossing over for each of the five regions calculated and listed in the columns at the right. The successive percentages of crossing over for each of the five regions are plotted as curves in figure 1, the dotted line in each case being the experimental value and the full line the control. The results of the tests of the multiple third chromosome stock are given in tables 2 and 3, with the percentages of crossing over in the columns headed per cent 1, etc. Table 2 summarizes the results of a ten-day brood count made with the sepia-Dichete-spineless-sooty-rough stock, and table 3 shows the three-day-interval results from the same stock without Dichete. The crossover values of table 3 are plotted as curves in figure 2.

INTERPRETATION OF THE CURVES OF CROSSING OVER

An examination of the curves in figure 1 demonstrates the following facts. First, the full and dotted lines for each of the five regions show no significant differences. The work on the second chromosome showed that for a region that was sensitive to temperature, the dotted line was significantly higher than the

TABLE 1

Summaries of first chromosome series

sc ec ee ct v g f

DAYS AFTER MATING	NON CROSS-OVER	1	2	3	4	5	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5	TRIPLES			TOTAL	PER CENT 1	PER CENT 2	PER CENT 3	PER CENT 4	PER CENT 5
Control—24°C. continuously																									
1-4	343	42	84	81	59	64	8	7	8	9	7	8	13	1	3	0				737	10.0	16.4	13.7	10.3	12.3
4-7	232	22	64	55	44	56	0	4	7	3	1	6	7	1	3	0	(2-4-5) 1			506	07.1	15.6	12.6	11.6	13.8
7-10	221	17	53	49	43	43	1	4	8	4	2	7	5	3	7	1	(3-4-5) 1			469	07.2	14.5	14.3	13.4	13.0
10-17	162	25	49	46	25	49	0	3	6	3	0	6	8	1	1	0	(1-4-5) 1			385	09.6	16.4	13.2	10.1	16.1
Heat treated—hatched at 31.5°C., mated at 24°C.																									
1-4	139	15	41	37	28	23	0	0	1	3	2	7	2	0	5	0	(1-2-5) 1			306	06.8	17.6	15.0	12.1	11.4
4-7	175	10	36	28	22	27	0	1	2	4	6	3	1	2	1	1	(1-2-3) 1			319	05.3	14.4	11.9	09.4	10.7
7-10	358	25	91	78	62	57	4	12	5	11	4	9	11	4	12	2	(1-3-5) 1			747	07.7	15.9	14.9	11.1	12.8
10-13	206	17	45	41	36	27	4	8	10	7	1	2	9	1	0	1	(3-4-5) 1			415	11.1	14.7	12.3	12.0	10.6
13-17	128	9	42	34	18	31	0	3	6	5	1	8	11	0	3	3				302	07.6	20.5	13.6	15.2	13.9
17-24	189	18	59	59	48	39	0	2	1	6	1	4	7	0	2	2	(2-3-5) 1			438	06.2	16.4	14.8	12.8	13.0
24-31	63	4	11	13	8	8	0	0	2	5	0	3	3	0	2	0	(3-4-5) 1			122	09.0	13.9	12.3	10.8	14.7
Summaries of first chromosome ten-day brood counts—24°C. continuously																									
	796	81	201	185	146	163	9	15	23	16	10	21	25	5	13	1	(2-4-5) 1	(3-4-5) 1		1712	08.4	15.6	13.4	11.6	12.8
Coincidence							0.40	0.78	1.38	0.86	0.26	0.71	0.75	0.22	0.47	0.16									

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full line for the first eight or ten days. It later coincided with the control after all the eggs were laid which had passed the critical period—when crossing over probably occurs—at the high temperature. No section of the first chromosome tested, therefore, shows any effect of high temperature on the amount of crossing over. This is a complete confirmation of my earlier conclusion, made on less exact data, and for a much shorter

Fig. 1   Curves of crossing over for different regions of chromosome I. The control values are the solid lines; the values from the heat-treated lines are dotted. The abscissas are days after mating, the ordinates percentages of crossing over.

section of the chromosome. Second, a comparison of the different control lines with each other indicates no significant variation as the female grows older. The control line for second chromosome regions dropped steadily up to about the tenth day, and gradually rose up to about the twenty-second. The sex chromosome control values show no significant nor uniform changes, and confirm the conclusion of Bridges ('15) that in this chromosome the age of the female has no influence on the amount of crossing over.

TABLE 2

Preliminary test of chromosome III, ten-day broods

se   ss   e^s   ro
D

Control—22°C. continuously

0	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-1	1-2-3	1-2-4	1-3-4	2-3-4	TOTAL	PER CENT 1	PER CENT 2	PER CENT 3	PER CENT 4
266	30	48	49	70	10	6	12	7	17	6	0	2	0	2	525	11.4	16.4	13.3	20.8
Coincidence......1.22							0.78			0.55									

Hatched at 31.5°C., mated at 22°C.

0	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-1	1-2-3	1-2-4	1-3-4	2-3-4	TOTAL	PER CENT 1	PER CENT 2	PER CENT 3	PER CENT 4
103	15	63	19	31	9	8	9	13	15	6	3	8	1	2	305	17.3	33.8	14.9	23.6
Coincidence......1.02							0.99			0.78									

TABLE 3

Summaries of third chromosome series

se   ss   e^s   ro

DAYS AFTER MATING	NON CROSSOVER	1	2	3	1-2	1-3	2-3	1-2-3	TOTAL	PER CENT 1	PER CENT 2	PER CENT 3	COINCIDENCE 1-2	COINCIDENCE 1-3	COINCIDENCE 2-3
Control—24°C. continuously															
1-4	337	129	47	96	26	40	2	0	677	28.7	11.0	18.9	1.20	1.09	0.14
4-7	493	180	97	154	32	49	1	2	1008	26.1	13.1	20.4	0.93	0.90	0.04
7-10	507	183	82	136	25	45	1	0	979	25.7	10.9	18.5	0.89	0.95	0.05
10-13	455	157	72	126	30	27	3	2	872	24.7	12.1	18.0	1.14	0.69	0.14
13-16	506	198	95	123	24	53	8	0	1004	27.4	12.6	18.3	0.69	1.08	0.47
16-19	380	146	71	93	11	28	0	3	732	25.6	11.6	16.9	0.55	0.87	0.30
19-26	185	74	25	66	11	18	0	0	379	27.1	09.5	22.1	1.13	0.79	
Heat treated—hatched at 31.5°C., mated at 24°C.															
1-4	195	97	34	54	20	31	3	1	435	34.2	13.3	20.4	1.01	1.02	0.25
4-7	370	228	67	140	37	86	11	10	948	39.0	13.1	26.6	0.76	0.94	0.31
7-10	257	130	51	76	29	37	6	1	587	33.5	14.8	20.4	0.99	0.92	0.33
10-13	257	96	45	78	15	23	4	0	518	25.8	12.4	20.2	0.90	0.84	0.32
13-16	212	92	36	55	16	22	2	1	436	30.0	12.6	18.3	0.98	0.91	0.21
16-19	208	98	41	67	10	20	1	1	446	28.8	11.8	19.9	0.65	0.78	0.09
19-26	200	81	34	49	6	14	2	1	387	26.2	11.1	16.9	0.52	0.79	0.27

An examination of tables 2 and 3 and of figure 2 discloses an interesting situation in the third chromosome. It seems clear from table 2 that high temperature causes a definite increase in crossing over between sepia and Dichete and a very marked one between Dichete and spineless, but little if any change in the remainder of the chromosome. Table 3 and a comparison of the full and dotted lines in figure 2 bring out this fact even more definitely, but without separating sepia and spineless by the
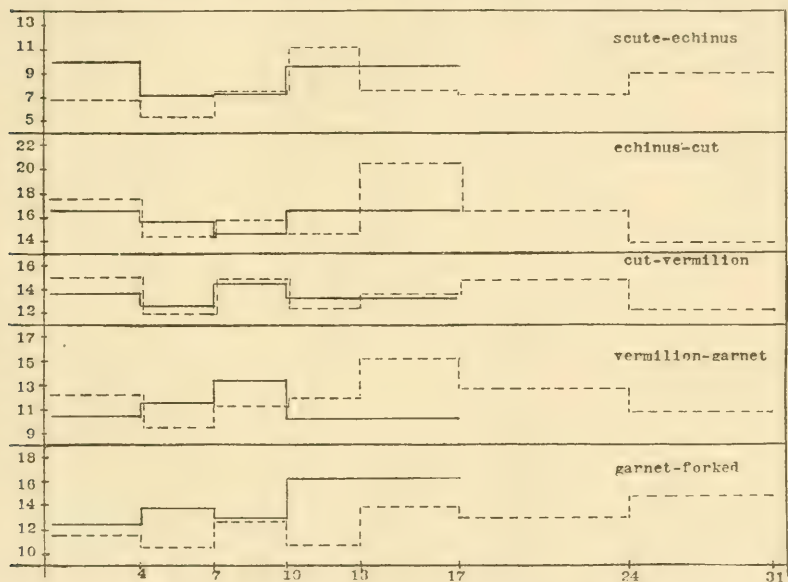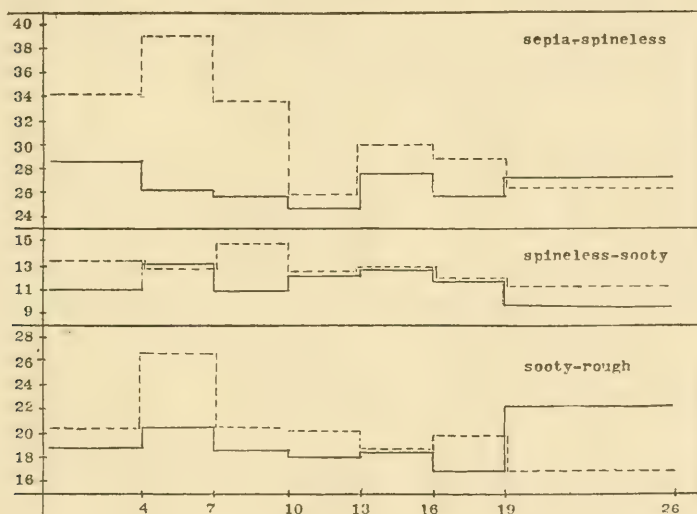

Fig. 2   Curves of crossing over for different regions of chromosome III. The control values are the solid lines; the values for the heat-treated lines are dotted. The abscissas are days after mating, the ordinates percentages of crossing over.

Dichete factor. The dotted line for the sepia spineless region begins at a point about 6 units higher than the control, rises to a difference of 13 units, and drops sharply to about the same point at about the tenth day. This indicates, as in the second chromosome, that the eggs which go through the critical period at the high temperature show a much increased crossover ratio, but that those which pass through that period subsequently (i.e., after the females are replaced at the control temperature) are not affected. The dotted line for the spineless sooty region, on the other hand, shows no significant difference. That for

sooty rough shows a rise of nearly six units in the four-to-seven-day period, but since no difference appears either before or after this time, it is probable that this has no significance. The data indicate, therefore, that the percentage of crossing over is increased by exposure to high temperature at one end of chromosome III, but not throughout the remainder of its length.

It is interesting to note that the control line for the sepia spineless region shows the age variation observed in the second chromosome. The value gradually drops to the tenth day and then rises. The rise apparently takes place somewhat earlier than in chromosome II. The other two regions show no significant difference as the female grows older.

The results of the tests may be summarized as follows: a) the sex chromosome shows no significant increase in the percentage of crossing over as a result of the exposure of the developing eggs to high temperature; b) the third chromosome shows an increase in crossing over in the sepia spineless region, but nowhere else; c) a variation in crossing over with the age of the female occurs in those regions which show a reaction to temperature only.

REACTION TO TEMPERATURE AND HIGH COINCIDENCE

In figure 3 I have drawn to the same scale comparative maps of the principal chromosome regions whose reactions to high temperature have been tested. The percentages of crossing over in chromosome I have been calculated from the ten-day brood counts summarized at the end of table 1, and those for chromosome III from table 2. The map of chromosome II is taken (for the points indicated) directly from the very accurate one given by Bridges and Morgan (p. 302). The regions for which a rise in the percentage of crossing over as a result of exposure to a temperature of 31.5°C. has been recorded—either in this or my former paper—are indicated by diagonal lines, while those which are not changed are solid black. As noted previously, it may develop that one or both of the long regions at either end of chromosome II will show a reaction to temperature if they can be broken into short blocks. A rise in total crossing over may be obscured by a compensating rise in double

crossing over, so that no result appears in the count.   With the exception of these two regions, in which Bridges ('15), Plough ('17), and Bridges and Morgan ('19) have recorded an age differ-ence, the diagonal lines also indicate the regions which show a variation in crossing over with age.
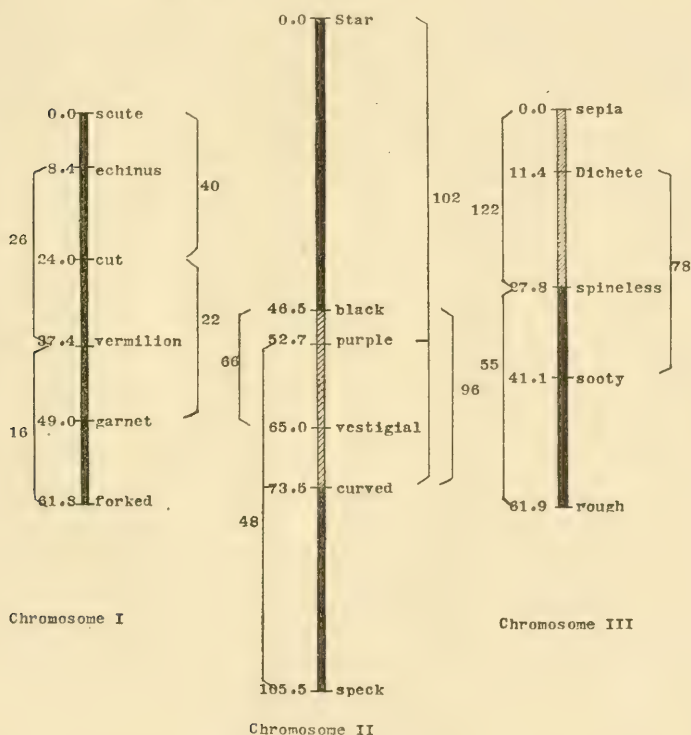
Fig. 3   Chromosome maps showing all the important regions whose reaction to high temperature has been tested.   The regions which show a significant in-crease in crossing over after exposure to high temperature are ruled with diag-onal lines those not affected are solid black.   The coincidence values are given outside of brackets enclosing the different pairs of adjacent regions.

I have recently laid some emphasis on the fact that in the sensitive section of chromosome II the percentage of increase in crossing over due to high temperature was roughly in inverse proportion to the length of the region involved (Plough, '19). The results in chromosome III demonstrate very plainly, however,

that the masking effect of increased double crossing over is not the only reason why certain regions remain unchanged after exposure to high temperature. Table 2 shows that in chromosome III the Dichete spineless region (16.4 units) shows an increase of more than 100 per cent, while sepia Dichete (11.4 units) is increased only about 50 per cent, and spineless sooty (13.3 units) is practically unchanged. It is apparent that there are other factors than the mere length of the region responsible for the difference in reaction to high temperature. Not only are different chromosomes unlike, but within each chromosome certain regions show distinct differences in behavior from certain other regions. This fact has been apparent to several Drosophila workers in connection with the investigation of the coincidence values, and has been the subject of a special study by Bridges, the results of which are not yet published. It is of some interest to compare the differences in reaction to temperature with the coincidence results.

Figure 3 shows also the coincidence of double crossing over for each pair of adjacent regions as a percentage value outside a bracket enclosing the two regions for which it has been calculated. The significance of the value for coincidence has been discussed in detail by Bridges ('15), Muller ('16), Weinstein ('18), and Bridges and Morgan ('19). It represents the percentage of the expected number of double crossovers actually observed. The size of the coincidence value has been shown by a number of observers to be in proportion, up to a certain point at least, to the distance apart of the opposite boundaries of the regions tested (i.e., to the lack of interference). For instance, a glance at the ten-day counts in table 1 shows that the coincidence value is high when the scute-echinus and vermilion-garnet regions (138 per cent) or the scute-echinus and garnet-forked regions (86 per cent) are figured, but low when the regions are closer together.

A comparison of the coincidence values given for approximately equal lengths of chromosome shows that they do not correspond. Double crossing over per unit of distance is apparently much

more frequent in certain regions than in others, and this is true not only when the regions compared are in different chromosomes, but when they are in different portions of the same chromosome. For instance, for the black-purple-vestigial region of chromosome II we get a coincidence of 66 per cent,[1] while for distances slightly longer in chromosome I-scute to cut or cut to garnet—the values are 40 per cent and 22 per cent, respectively. The black-purple-curved region in chromosome II gives a coincidence value of 96 per cent (Plough, '17, table 8), while a similar length in chromosome I—echinus-cut-vermilion—gives 26 per cent. Even more striking, however, is the fact that the region at the upper end of the third chromosome on the map—sepia-Dichete-spineless, a distance of 28 units—gives a coincidence value of 122 per cent, yet the longer lower region—spineless-sooty-rough (33 units) gives only 55 per cent coincidence. This indicates that double crossing over is at least as frequent as though there were no interference at the sepia end of chromosome II, but interference is almost as high at the rough end as in chromosome I. A difference of the same order is apparent in chromosomes I and II.

It will now be obvious that those sections of the chromosomes mapped in figure 3 which show a relatively high coincidence per unit of distance are the same ones which show a change in the amount of crossing over as a result of high temperature and of the age of the female. In no case where the coincidence value for a continuous region of 30 units or less is below about 60 per cent do we find an increase in crossing over due to high temperature, or, with the possible exceptions noted in the second chromosome, a change due to age. The chromosomal regions which are 'sensitive' to environmental effects all show a minimum of influence of one crossover on another simultaneous crossover in the same region.

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[1] Bridges and Morgan, '19, table 42—not 61 per cent, as they give it.

DISCUSSION

The fact that high coincidence and sensitiveness to environmental effects are found in the same chromosomal regions suggests that certain structural features of the crossing over process determine each. Bridges and Morgan (p. 188) suggest that the difference in the amount of interference for short regions between the first and the second chromosomes may be interpreted in two ways. We may assume that the average length of loop between simultaneous crossovers is the same in each, which means that a region having a given coincidence value in chromosome II is actually the same length as one having the same coincidence in chromosome I. On the other hand, we may hold that the length of loop between simultaneous crossovers is relatively shorter in chromosome II than in chromosome I, which means that equal amounts of crossing over then indicate equal lengths of chromosome. Either of these alternatives holds also for the different sections of chromosome III. According to the former interpretation, crossing over takes place relatively less freely in the regions ruled with the diagonal lines and they are actually much longer than the map length indicates. According to the latter view, crossing over takes place relatively more freely, and the map lengths are accurate. Bridges ('19, and from subsequent unpublished data) distinctly favors the former interpretation. The effect of high temperature in causing an increase in these regions does not give any clear evidence for either view, though it would seem to support Bridges' interpretation. It is hardly possible that temperature does not act on the whole chromosome equally. Any observed differences between different regions would seem to be due to the fact that slight effects are registered in certain regions and not in others. It is reasonable to suppose that the regions in which a change is observable should be those in which crossing over is less free.

It is of some interest to consider what structural conditions in the chromosomes could result in regions of decreased freedom of crossing over. Bridges and Morgan (p. 198) and Bridges ('19) suggest that the reason for the difference in behavior of the black curved region in chromosome II may lie in the fact that this

region is near the middle of the chromosome, "with the spindle fiber attachment, and that this middle region is the last part to undergo synapsis." Bridges has subsequently applied this same idea to chromosome III and decided its middle point is close to the locus for Dichete. In the latter case the conclusion as to the midpoint of the chromosome has been definitely confirmed with the finding by Strong ('20) of the locus for roughoid at 24.9 units beyond sepia. If, as Bridges suggests, crossing over is less in this middle region because synapsis fails or is slight, the decreased freedom of crossing over might be consistently explained. On the other hand, it should be definitely borne in mind that such behavior is an observable phenomenon, which is susceptible of cytological demonstration. The demonstration that the process of crossing over is accomplished by a simple twisting separation, and reunion of chromosome strands is still incomplete, and we have no cytological data which indicate that in Drosophila the middle region is the last part to undergo synapsis. At the early stage in the growth period of the egg at which crossing over apparently takes place it seems altogether unlikely that the spindle fiber suggested by Bridges is present at all. Until we know more of the actual cytological features of the crossing-over process and of the spindle fiber attachments in Drosophila, such suggestions must be regarded as highly speculative.

AGE AND TEMPERATURE EFFECTS COMPARED

It has been demonstrated above that in general both age and temperature affect the amount of crossing over in the same chromosome regions—those probably in which there is a minimum of crossing over. It is to be expected, therefore, that the freedom of crossing over is modified by both agents. It is of some interest to note that Bridges and Morgan (p. 199) and also Bridges ('19) in identical language conclude that the age variation is probably due "to a lengthening of the average length of the section of chromosome between simultaneous crossovers," while temperature causes an increase in the freedom of crossing over with no difference in the length of loop. The clearest evidence

for this conclusion is stated to be found in a calculation of the coincidence values for my two-day-interval experiment for the black-purple-curved region reported in my former paper. They state (p. 199):

> In this experiment the curve of variation in coincidence was the mirror image of the curve of variation (in crossing over) for age. The curve of coincidence corresponding to the curve of temperature variation found by Plough seems to be a straight line cutting the rises and falls of the temperature curve and independent of them.

TABLE 4

Coincidence values for control and heat-treated lines in two-day-interval experiment. (For actual counts cf. Plough, '17, table 14)

b   pr   c

NUMBER DAYS AFTER MATING	CONTROL—22°C. CONTINUOUSLY		PARENTS HATCHED AT 22°C., EXPOSED TO 31.5°C. FROM 3RD TO 11TH DAY AFTER MATING	
	Per cent of crossing over—b, pr region	Coincidence b, pr–pr, c	Coincidence b, pr–pr, c	Per cent of crossing over—b, pr region
3	8.3	0.91	1.01	7.1
5	4.9	0.94	1.31	4.8
7	6.8	1.13	0.53	3.8
9	5.8	1.03	1.37	3.8
11	4.2	1.06	0.63	8.8
13	5.1	0.80	0.94	13.9
15	5.3	1.40	0.99	19.2
17	4.2	0.92	0.95	20.0
19	7.3	0.93	0.73	17.5
21	8.2	1.04	1.57	6.8
23	7.9	0.63	0.27	4.9
25	7.0	0.98		1.4

I have calculated the coincidence values for the experiment cited and the results are given in table 4. A comparison of my coincidence values with the crossover percentages for the black-purple region shows that the coincidence value varies within rather wide limits in both the control and experimental lines. A smoothed curve gives some suggestion of the relation claimed by the writers quoted, but its significance is doubtful. The same comparison may be made between the similar lines in chromosome III (table 3). The coincidence values for the different pairs of regions are given in the last three columns. Here there

is surely no mirror-image relation in the control series. In
addition, the coincidence is subject to so high a probable error
that it would take very marked and constant differences to
establish such a conclusion as the one stated. It seems clear
that much weightier evidence than that quoted must be given
before it can be established that age and temperature act in
different ways on the crossing-over process. It is more con-
sistent with the results here given that each causes a variation
in the actual freedom of crossing over and that the changes in
coincidence recorded are without significance.

SUMMARY

1. It has been shown that a temperature of 31.5°C. causes
little or no effect on crossing over in any part of the sex chromo-
some, nor is there any significant variation with the age of the
female.

2. Crossing over in the sepia-spineless region of chromosome
III is increased by a temperature of 31.5°C., the effect being
most marked between Dichete and spineless.

3. The same region shows a variation in crossing over with
the age of the female parent.

4. Crossing over in the remainder of chromosome III is influ-
enced neither by temperature nor age.

5. The chromosomal regions which are 'sensitive' to temper-
ature and to age all give a very high ratio of double to single
crossing over.

6. This is interpreted as indicating that the effects of environ-
ment cause observable differences in crossing over only where
crossing over occurs least freely.

7. It is shown that the view that temperature and age act
on crossing over in different ways is not established.

LITERATURE CITED

BRIDGES, C. B.   1915   A linkage variation in Drosophila.   Jour. Exp. Zoöl.,
        vol. 19, no. 1, July.
        1919   The genetics of purple eye color in Drosophila.   Jour. Exp. Zoöl.,
        vol. 28, no. 2, May.
BRIDGES, C. B., AND MORGAN, T. H.   1919   The second chromosome group of
        mutant factors.   Publ. no. 278, Carnegie Inst. Wash.
MULLER, H. J.   1916   The mechanism of crossing over.   Amer. Nat., vol. 50.
PLOUGH, H. H.   1917   The effect of temperature on crossing over.   Jour. Exp.
        Zoöl., vol. 24, no. 2, November.
        1919   Linear arrangement of genes and double crossing over.   Proc.
        Nat. Acad. Sci. U. S., vol. 5, May.
STRONG, L. C.   1920   Roughoid, a mutant located to the left of sepia.   Biol.
        Bull., vol. 38, no. 1, January.
WEINSTEIN, A.   1918   Coincidence of crossing over in Drosophila.   Genetics, 3,
        March.

Resumen por los autores, William E. Burge
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Una explicación de la variación en la intensidad de la oxidación
durante el ciclo vital.

Es un hecho conocido que la oxidación o metabolismo es muy
baja en el óvulo no fecundado, mientras que aumenta notable-
mente a raíz del proceso de la fecundación; que el metabolismo
del niño recién nacido es también muy bajo, pero que aumenta
rápidamente llegando a ser muy elevado durante la niñez, dis-
minuyendo después gradualmente desde la edad adulta hasta la
vejez. Los autores han observado que 0.5 gramos de los huevos
no fecundados de Leptinotarsa, macerados, desprenden 18 cc.
de oxígeno en diez minutos cuando se tratan con peróxido de
hidrógeno, y que 0.5 gramos de huevos fecundados desprenden
35 cc. durante el mismo tiempo. 0.5 gramos de larvas recién
salidas del huevo, durante la cuarta parte, mitad, tres cuartas
partes del desarrollo y larvas completamente desarrolladas
desprenden 280, 800, 1250, 1725 y 1750 cc., respectivamente,
y que las ninfas, adultos e individuos viejos desprenden 1800,
1750 y 900 cc. de oxígeno, respectivamente. Comparando estas
figuras puede comprobarse que el huevo no fecundado contiene
mucha menos catalasa que el fecundado; que el contenido de
catalasa en las larvas recién salidas del huevo es menor que el
de las larvas más avanzadas y que el contenido de catalasa en
el individuo viejo es menor que el del adulto más jóven.

La reducida cantidad de oxidación en el huevo no fecundado
se debe probablemente a su escaso contenido de catalasa. La
oxidación aumentada del huevo fecundado y su desarrollo consi-
guiente se atribuyen a un aumento de catalasa introducida por
la estimulación del huevo para la producción mayor de esta
enzima por parte del espermatozoide. Del mismo modo el
aumento del metabolismo respiratorio u oxidación en el jóven
y su disminución con la edad avanzada, se atribuyen a un au-
mento de catalasa en el jóven y a su disminución en el de más
edad.

AN EXPLANATION FOR THE VARIATIONS IN THE INTENSITY OF OXIDATION IN THE LIFE-CYCLE

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ONE FIGURE

As a result of the work of a great number of observers, particularly of Hasselbalch (*1*), Magnus-Levy and Falk (*2*), and of Warburg (*3*), it is now known that oxidation or metabolism is very low in the unfertilized ovum, while it increases greatly following the process of fertilization; that the metabolism of the newly born infant also is very low, but increases rapidly, becoming very high during childhood and then gradually decreasing from maturity to old age. The present investigation is an attempt to find an explanation for the variation in the intensity of oxidation under the conditions named.

Since we (*4*) had found that whatever increased oxidation in the body, the ingestion of food, for example, produced an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide, by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation, narcotics, for example, diminished catalase by decreasing its output from the liver and by direct destruction, we naturally turned to catalase in seeking an explanation for the variations in the intensity of oxidation at different periods in the life-cycle.

The Colorado potato beetle (Leptinotarsa decemlineata) was used in this investigation. Catalase determinations were made of the following materials gound up in a mortar: unfertilized and fertilized eggs, quarter, half, three-quarter, and full-grown larvae, as well as pupae, adult, and very old beetles. Five-tenths gram of the ground material were added to neutral hydro-

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gen peroxide in a bottle and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase content of the material.

AMOUNTS OF CATALASE MEASURED IN CC. OF OXYGEN.

UNFERTILIZED EGG	18
FERTILIZED EGG	35
NEWLY HATCHED LARVA	280
QUARTER GROWN LARVA	800
HALF GROWN LARVA	1250
THREE QUARTER GROWN LARVA	1725
FULL GROWN LARVA	1750
PUPA	1800
ADULT BEETLE	1750
OLD BEETLE	900

Fig. 1 The figures in the chart indicate amounts of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 gram of the material ground in a mortar.

The results of the determinations as well as photographs of the beetles, pupae, larvae, and eggs are shown in figure 1. It may be seen that 0.5 gram of the unfertilized eggs liberated 18 cc. of oxygen in ten minutes from hydrogen peroxide and 0.5 gram of the fertilized eggs, 35 cc.: that 0.5 gram of the newly hatched, quarter, half, three-quarter, and full-grown larvae

liberated 280, 800, 1250, 1725, and 1750 cc., and that the pupae, adult, and old bugs liberated 1800, 1750, and 900 cc. of oxygen, respectively.

By comparing these figures it may be seen that the unfertilized egg contains much less catalase than the fertilized. This is in keeping with the fact that the oxidative processes are much less intense in the unfertilized egg than in the fertilized one, as observed by Warburg. It may be seen further that the catalase content of a newly hatched larva is less than that of the older larvae in keeping with the fact that in the newly born, and presumably in the newly hatched, oxidation is very low and that it increases very rapidly shortly after birth. It may also be seen that the catalase content of the old bug is less than that of the younger adult in accordance with the fact that oxidation or metabolism is less in a person of advanced age than in one in middle life.

It should be mentioned in this connection that our observation of the low catalase content of the unfertilized potato-beetle egg and the high catalase content of the fertilized egg is in keeping with the observation of Winternitz (5), who found that the unfertilized hen's egg showed no catalytic activity even after prolonged incubation, whereas the incubated fertilized egg rapidly acquired the power of decomposing hydrogen peroxide. They agree also with the observations of Battelli and Stearn (6), who found that the catalase content of most of the tissues, and particularly of the liver, of newly born pigs is lower than the corresponding tissues of the mother, but that the catalase activity rapidly increased, until at the end of the seventh or eighth day it was as high as that of the adult.

J. Loeb (7) attributes the development of the fertilized sea-urchin egg to the increase in oxidation, and the increase in oxidation to a change in the cortex of the egg which makes the entrance of oxygen, and hence oxidation, possible, while R. Lillie (8) holds that the cortical layer of the unfertilized egg prevents the diffusion of CO_2 from the egg and that this CO_2 prevents oxidation, and hence development. A more plausible explanation for the increased oxidation or metabolism in the

fertilized egg, and hence for the development of the egg, would seem to be that the spermatozoon furnishes a substance which stimulates the egg to an increased formation of catalase. Further evidence that might be presented in support of this view is afforded by the fact that the very same chemicals (amines, alkalies, acetates, butyric acid, etc.) which Loeb found would bring about increased oxidation and artificial parthenogenetic development of the egg, we found, when introduced into the alimentary tract of animals, stimulated the alimentary glands, particularly the liver, to an increased output of catalase with resulting increase in oxidation.

SUMMARY

The low rate of oxidation in the unfertilized ovum is attributed to its low catalase content. The increased oxidation in the fertilized ovum, with resulting development, is attributed to an increase in catalase brought about by the stimulation of the egg to an augumented production of this enzyme by the spermatozoon.

Similarly, the increase in the respiratory metabolism or oxidation in youth and decrease in old age is attributed to the increase in catalase in the young and its decrease in the aged.

BIBLIOGRAPHY

1 HASSELBALCH 1904 Bibliotek for laeger, Copenhagen, vol. 8, p. 219.
2 MAGNUS-LEVY UND FALK 1899 Arch. f. Anat. u. Physiol., Suppl. 315.
3 WARBURG 1908 Zeitschr. f. physiol. Chem., Bd. 57, No. 6.
4 BURGE 1918 Am. Jour. Physiol., vol. 45, no. 4; vol. 47, nos. 1 and 3.
5 WINTERNITZ AND ROGERS 1910 Jour. Exper. Med., vol. 12, no. 12.
6 BATTELLI E STEARN 1905 Arch. di Fisiol., T. 2, p. 471.
7 LOEB 1913 Artificial parthenogenesis and fertilization. University of Chicago Press.
8 LILLIE 1910 Am. Jour. Physiol., vol. 27, p. 289.

Resumen por los autores, Henry Laurens y S. R. Detwiler,
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Estudios sobre la retina.

La estructura de la retina de Alligator mississipiensis y sus cambios fotomecánicos.

El ojo de Alligator posee un tapetum retinal bien desarrollado, formado por la inclusión de guanina en las células epiteliales de las porciones dorsal y posterior, a una distancia de 1.5 mm. de la entrada del nervio óptico. El pecten consiste en una especie de copa pigmentada ligeramente elevada, que cubre la entrada del nervio óptico. En toda la retina se encuentran conos y bastones, pero la proporción de ambos es diferente en distintas regiones. Los bastones son todos del mismo tipo, los conos de dos tipos: grueso y delgado. Los primeros son más numerosos, presentándose especialmente en las regiones posterior y ventral de la retina. Los conos del segundo tipo se encuentran solamente en la porción ventral y no son numerosos. También existen conos dobles. Ninguno de los conos y bastones contiene gotas de grasa.

Los núcleos de los bastones son de forma oval y la mayor parte de ellos se proyectan a través de la membrana limitante externa en una extensión variable. Los núcleos de los conos son piriformes y ocupan un nivel más profundo que el de los núcleos de los bastones, formando una segunda fila. Los bastones presentan un cambio de longitud media de unas 4 micras, y son más largos a la luz. Los conos sencillos presentan un cambio medio de 2.1 micras y son más cortos a la luz. Los miembros mas pequeños de los conos dobles presentan un cambio medio de longitud de 3.5 micras, y los mayores de 2.7 micras. La emigración del pigmento es ligera, y su media es 1.6 micras, pero cuando se combina con el cambio de longitud de las células visuales produce una emigración efectiva igual a su suma. El trabajo termina con consideraciones teóricas sobre los cambios fotomecánicos y la teoría de la duplicidad bajo un punto de vista comparado.

STUDIES ON THE RETINA

THE STRUCTURE OF THE RETINA OF ALLIGATOR MISSISSIPPIENSIS AND ITS PHOTOMECHANICAL CHANGES

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THIRTEEN FIGURES

INTRODUCTION

Owing to the variation in kind and distribution of the visual cells, the reptilian retina offers an interesting field of investigation in structure and function, with particular reference to the probable functions of the rods and cones under diurnal and nocturnal conditions (duplicity theory). Detwiler ('16) studied the structure and photomechanical changes in the retina of a number of turtles and of lizards, and with the idea of making another contribution to our knowledge of the conditions holding in the reptiles the present investigation was undertaken. The work was started in the spring of 1917, but was necessarily abandoned, and only recently has it been possible to resume it.

The literature on the crocodilian eye is small and rather unsatisfactory. Heinemann ('77), in a study of the eyes of Mexican reptiles, described the visual cells of Crocodilus rhombifer cuv. as consisting of characteristic rods alternating with considerably fewer and shorter cones. In addition to the typical rods he found a much less numerous kind, with very long outer segments of platelet structure. The cones are described as being also of two kinds, thick and slender, neither of which contain colored oil drops, and which occur singly and together to form double cones. There is an ellipsoid with a small central body in the peripheral portion of the inner segment of both and

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a paraboloid in the slender cones. He could distinguish the cone nuclei from the rod nuclei by their more spherical form and larger size, but describes both as occupying a single layer.

Tafani ('83) studied the retina of the crocodile, Champsia lucius, and found rods to be the predominant type of visual cell. In the anterior part of the retina there are practically no cones, but as the fovea is approached they become gradually more numerous than the rods. He found, unlike Heinemann, only one kind of rod. He describes the cones as being short, with a barrel-shaped inner segment, but with an outer segment similar to that of the rods, although his figures do not bear out his description. The differences between the nuclei of the cones and of the rods, which occur in a single layer, he considers too slight and inconstant to be considered as of any significance.

Chievitz ('89) described in some detail the pigmented epithelium and the tapetum. In the eye of Alligator mississippiensis the tapetum extends through the entire upper half of the retina in the form of a bright band, reaching nearly to the ora, while its lower margin lies about 2 mm. above the entrance of the optic nerve in a 32-cm. specimen. In this bright band he found a fovea in the form of a very superficial, narrow furrow with thickened edges, and running horizontally across the entire tapetum about 1 mm. from its lower edge. He could not decide whether rods as well as cones occurred. In a vertical section of the eye of Crocodilus intermedius the tapetum is seen as a longitudinal bright stripe in the middle of the pigmented epithelium. In this region the middle part of the epithelial cells contain a number of fine, whitish, opaque granules of guanin, which when removed leave the middle portion of the cells colorless, while the choroidal and the vitreal portions contain melanin. The nucleus lies in the guanin-containing portion, directly against the basal pigment. At the margin of the tapetum black pigment is present in almost the entire cell; toward the middle, the vitreal pigment is gradually reduced and eventually exists only in the form of isolated, iregularly distributed small masses, between which the guanin comes to the edge of the cells. In the alligator the pigment in the choroidal portion of the cells is sparse and

the nuclei are very close to the basal cell boundary. The pigment processes reach as far as the inner segments of the visual cells, the outer segments being deeply imbedded in the guanincontaining portion of the epithelium.

Krause ('93) described the visual cells in the retina of Alligator mississippiensis and quotes from Hofmann's description of the retina of Crocodilus vulgaris. In the alligator, Krause considers that the rods could be taken for small cones, because the slender inner segments are slightly tapering, while the outer segments are almost cylindrical. The inner segments of the cones, on the other hand, are thick and the outer segments short and pointed. Hofmann describes the rods of the crocodile as numerous except in the fovea and the surrounding regions of the retina. They are very similar to the red rods of the frog, and Krause reproduces a figure from Hofmann of a rod and cone. The cones are single and double. Krause reproduces (again from Hofmann) cones with very long, pointed outer segments from the fovea. There are no rods in the area and only single cones, the inner segments of which become narrower as the fovea is approached. According to Krause, the nuclei of the visual cells in Alligator mississippiensis are all in one row, the cone nuclei being rounder than those of the rods. In the crocodile, Hofmann says that they occupy two rows, with the rod nuclei next to the external limiting membrane.

Abelsdorff ('98) considers that very strong support is given by the conditions in the reptilian retina to the view first put forth by Max Schultze, that the rods serve for the reception of weak and colorless light stimuli. He calls attention to the fact that most reptiles have practically only cones, the exceptions being the geckos (in some of which the cones seem to be entirely lacking), the crocodiles, and the boa. These are all nocturnal animals. The crocodile, he says, on account of its rod-rich retina, is not only capable of seeing in a very weak light, but can find its way about in pitch darkness, this property being enhanced by the light reflecting tapetum in the upper portion of the eye, the rods being thereby doubly stimulated. Abelsdorff points out that it is particularly in water that the upper part of the eye

needs an increase in intensity of the light impression more than does the lower part of the eye, because the upper portion receives only what little light may be reflected from the bottom.

He figures the tapetum in a sagittal section where it can be seen in the upper portion of the eye between the choroid and retina proper, going over in the lower portion of the eye, with a gradually increasingly thick black border, into the guanin-free, melanin-containing portion of the pigmented epithelium. Attempts to demonstrate a change in position of this pigment in light and darkness were unsuccessful. It is interesting to note in this connection that Garten ('07, p. 89) considered it worth while to have this experiment repeated, which he did, with results (p. 108) substantiating those of Abelsdorff. Abelsdorff describes the rods of the alligator as being similar to those of frogs, but of narrower diameter. He found that the visual purple, investigated by direct observation of the opened eye as well as ophthalmologically (assisted therein by the presence of the tapetum), was not confined to the upper portion of the eye, but, by turning the retina over and looking at it from the visual cell side, could be seen as well in the lower portion. He investigated the bleaching of the purple in daylight as well as the relative amount of bleaching and the time relations upon exposure to light of various wave length. The fact that the purple was seen throughout the eye would seem to indicate that the rods occurred throughout the retina, although, if one chose to follow Edridge-Green, it might be assumed that the purple diffused into the regions where the rods were not present.

Finally, Garten ('07, p. 109) describes the visual cells in Alligator lucius. In the upper part of the retina (guanin portion) there are large cylindrical rods only, which are surrounded in the light as well as in the dark eye by a mantle of guanin. This part of the retina is absolutely cone free. In the lower portion of the retina the visual cells are relatively small, possessing a very thin tapering outer segment, which in light as well as in dark eyes is buried in pigment. Garten considers these to be all cones. He refers to Abelsdorff as having described visual purple in the lower portion of the retina, and thinks, since he

(Garten) has shown that there are only cones in this region, that this matter should be reinvestigated.

The conditions in the alligator eye Garten uses in substantiation of his conception of the functional value of the migration of pigment in connection with the movement of the visual cells. Since he finds the two parts of the retina containing exclusively cones or rods, he argues that pigment migration therefore should not take place, because the perceiving power of the eye would not thereby be in anyway enhanced. Garten calls attention to the importance of the fact that the conical visual cells go over very gradually into those of rod form, and points out that it is exactly at this transition place that Chievitz localized the fovea.

In two general reviews by Pütter ('12) and Franz ('13) the conditions found in the crocodilian retina are summarily given, but no new matter contributed. It should be recalled that Pütter is of the opinion that, although some of the elements in the reptilian retina may be cylindrical in form (that is rod-like), they are all nevertheless to be regarded functionally as cones, on account of their dendritic mode of connection with the bipolar cells.

METHODS

The alligators, which were between 45 and 55 cm. long, were treated as follows. Two animals were placed in a dark room for twenty-four hours, at the expiration of which time one of them was removed to bright diffuse daylight for seven hours. At the end of this time both were killed. The upper jaw, with the eyes, was removed with a pair of large bone forceps, bisected, and dropped into a large dish containing an abundance of Perenyi's fluid. The time required for this operation did not exceed thirty seconds, and in the dark was carried out in the weak light from a photographic lamp. The pupil of the alligator is vertical, and when the animals are placed in light the aperture remaining after a few seconds' exposure is but a mere slit. The pupillomotor reaction is so decisive, characteristic, and easily measurable, that experiments have been begun on the relative efficiency of spectral lights upon it. These will be reported later.

The halves of the upper jaw containing the eyes were allowed to remain in the fixing fluid for an hour, in light or darkness, respectively, without being disturbed. After the expiration of this time, they were carefully dissected out and dropped into
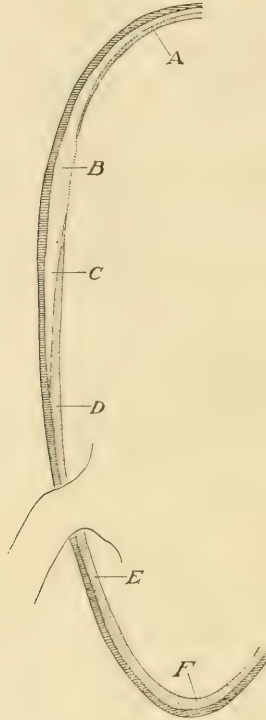
Fig. 1 Diagrammatic drawing of sagittal section of the epithelial pigment layer. The guanin is indicated by the lightly stippled, the melanin by the heavily stippled area; the choroid is shown by horizontal lines. $\times$ 11.

fresh fixative, where they remained from four to five hours longer. Sagittal and horizontal sections 10μ thick were made, stained in eosin and toluidin blue, iron haematoxylin and eosin, or in Ehrlich's haematoxylin and eosin. All methods yielded good results.

ANATOMICAL

Epithelial pigment layer. A retinal tapetum occurs in the dorsal and posterior portions of the retina to within 1.5 mm. of the entrance of the optic nerve. It is formed by the inclusion of guanin in the cells of the epithelial layer. The relative amount and distribution of the guanin and the ordinary melanin is shown in figure 1. In the region designated by B the epithelium is relatively devoid of melanin, which forms a narrow border of a

Fig. 2   A portion of the tapetum designated by the letter B (fig. 1), showing broad zone of guanin, a narrow vitreal border of melanin, and a few scattered needles of pigment near the choroidal margin.   × 665.

Fig. 3   A portion of the epithelial layer designated by letter C (fig. 1), showing choroidal guanin-containing portion and vitreal melanin-containing portion. × 665.

Fig. 4   A portion of the epithelial pigment layer corresponding to the region designated by letter D (fig. 1).   × 665.

few needles along the vitreal margin and occurs also as scattered needles here and there in the choroidal portion of the cells (fig. 2). As the optic nerve is approached, the amount of melanin gradually increases as the guanin decreases, until within about 1.5 mm. above the entrance of the optic nerve guanin is no longer found. The gradual increase in the amount of melanin as the optic nerve is approached is seen in figures 2, 3, and 4, which show in detail the condition as found at the levels B, C, and D in figure 1. Above the level B in figure 1 the guanin again gradually decreases in amount and the melanin shows a corresponding increase (level A, fig. 1).

The guanin is light grayish-brown in color (stained sections), finely granular and fairly uniformly distributed through the cell (fig. 2). In the tapetum (level B, fig. 1), the guanin-containing protoplasm, although covering over the outer segments of the visual cells, does not show the finger-like projections which so typify the melanin-containing portions of the epithelium (figs. 2, 3, and 4).

In the lower portion of the retina the epithelial layer is entirely devoid of guanin. Here the melanin, in the form of delicate brownish-black needles, occupies the entire cell body and finger-like processes of the cells which project over and embrace the outer segments of the visual cells. The nuclei of the epithelial layer are spherical and occupy the choroidal portion of the cell body. Light and darkness have no effect on their shape and position.

Visual cells. The retina of Alligator mississippiensis contains both rods and cones, differing in this respect from the retinae of turtles and lizards (Detwiler, '16). The two kinds of visual cells in the alligator are not uniformly distributed, the cone-rod ratio changing in different parts of the retina. Histological examination of the retina has yielded the significant fact that no portion is rod or cone free and that there is no gradual transition from the conical elements into rod-like forms, as Garten ('07) claims. There are, however, areas which predominate in rods as well as areas which contain only a few rods. Viewing the retina as a whole, it can be said with justice that it is characteristically a rod-retina.

Rods. The structure of the rod is uniform throughout the retina. It consists of an inner segment composed of a cylindrical myoid and an ellipsoid and a cylindrical outer segment (fig. 5). No rods with conical outer segments could be found. The rod nuclei are typically oval in shape and lie just beneath the external limiting membrane projecting above it for variable distances in both dark and light eyes, and thus form the outer part of the external nuclear layer.

Cones. There are two kinds of cones, of which the predominating type is a large thick visual element very similar to that

found in the turtle retina.   The inner segment consists of a
short broad myoid, a broader refractive paraboloid, and an
ellipsoid, while the outer segment is relatively short and conical
(fig. 5).   This type of cone is found particularly in the posterior
and ventral portion of the retina.   The second type of single
cone (not very numerous), which is found only in the ventral
portion of the retina, has a considerably longer myoid and a
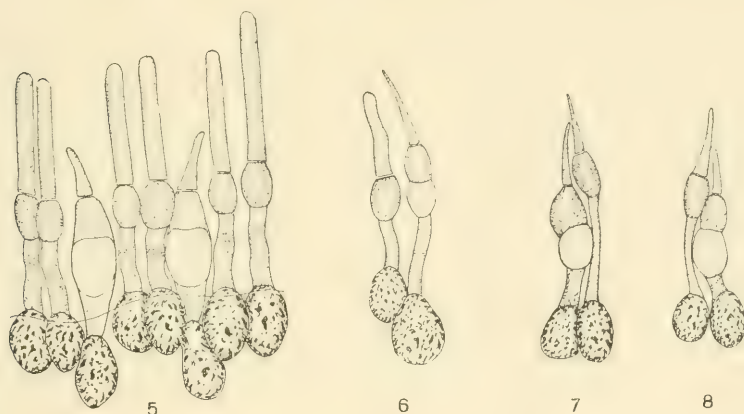
Fig. 5   A portion of the visual layer showing rods and large single cones.
× 935.   Drawing made at about 2.5 mm. above the entrance of the optic nerve
(level C, fig. 1).

Fig. 6   Elongated single cone and a neighboring rod taken from the ventral
portion of the retina.   × 935.   Animal in darkness for twenty-four hours.

Fig. 7   A double cone taken from the lower portion of the retina.   Animal
in darkness for twenty-four hours.   × 935.

Fig. 8   A double cone taken from the tapetal portion of the retina.   Animal
in diffuse light for seven hours.   × 935.

narrower paraboloid.   The conical outer segment is long, slender,
and pointed (fig. 6).

The double cones consist of a larger and a smaller member.
The former are very similar in shape to the large single cones.
The latter is characterized by a long slender myoid and the
absence of a paraboloid and has not been observed to occur
singly (figs. 7 and 8).   All cones lack oil drops, differing in this
respect from those of the turtle and lizard.

Relative distribution of rods and cones. In the upper peripheral portion of the retina there are very few cones. In the region designated by A (fig. 1), the ratio of rods to cones is about 95:5. The cones in this region, mostly single and of the large type, are irregularly distributed. In the typical tapetal portion (fig. 1, B) they are slightly more numerous (about 15 per cent). The majority of the cones, however, in this region are double, unlike those from the more peripheral portion of the retina. As the optic nerve is approached the number of cones shows a corresponding increase. In the region designated by C (fig. 1)

TABLE 1[1]

| | DISTANCE FROM NUCLEUS TO OUTER SEGMENT IN μ | | DISTANCE FROM NUCLEUS TO ELLIPSOID IN μ | | DISTANCE FROM NUCLEUS TO PARABOLOID IN μ | | | DISTANCE FROM ROD NUCLEUS TO NEAREST PIGMENT NEEDLE IN μ |
| | | | | | Single cones | Double cones | | |
	Cones	Rods	Cones	Rods		Large	Small	
Dark...............	19.8	13.5	14.5	7.5	6.8	6.7	13.0	11.5
Light..............	17.6	17.5	12.1	11.5	4.7	4.0	9.5	9.9
Difference........	2.2	4.0	2.4	4.0	2.1	2.7	3.5	1.6

[1] Total number of rod measurements,   280.
    Total number of cone measurements,   190.
    Total number of pigment measurements, 60.

Measurements made at about 2 to 3 mm. distance from the entrance of the optic nerve, in the region designated by C in figure 1, with the exception of the measurements of double cones which were taken from region B.

approximately 40 per cent of the visual cells are cones. Here the double cones are still more numerous than the single cones. In the guanin-free portion above the optic nerve (fig. 1, D) cones and rods are about equal in number, the single cones again exceeding in number the double. The cones outnumber the rods in the lower portion of the retina, the number increasing from the region of the optic nerve toward the ora serrata, and including all types. In this region only a few scattered rods are present. About 75 per cent of the cones in this region are single, the majority of which are like that shown in figure 5. The small type (fig. 6) is relatively scarce.

The cone nuclei are easily distinguished from the rod nuclei; the former being somewhat pear shaped, the latter more or less oval or elliptical.   Furthermore, the cone nuclei occupy a deeper level than the rod nuclei and constitute a second row.

<center>EXPERIMENTAL</center>

Effects of light: Rods and cones.   When sections of eyes of animals which have been exposed to diffuse light are compared with sections of eyes of animals kept in darkness, it is seen that there is an average difference in the lengths of the rod myoid of 4μ (table 1).   The relative lengths of the rod myoids in the dark and light conditions are shown in figures 9 and 10, as well as

<center>TABLE 2</center>

	DISTANCE FROM ROD NUCLEUS TO ELLIPSOID (MYOID) IN μ	
	Region	
	3 mm from optic nerve	Tapetum (region B, fig 1)
Light....................................	11.5	8.9
Dark.....................................	7.5	6.9
Difference............................	4.0	2.0

diagrammatically in figure 11.   The change in the length of the rods is found to be less extensive in the tapetal area (fig. 1, B), where rods predominate, than in the region close to the optic nerve, where the cones are considerably more numerous (table 2).

The effect of light on the cones is not so easily demonstrable. The results, however, of several series of measurements (table 1) show that the cone myoids of light eyes are slightly shorter (2.1μ) than those of dark eyes (figs. 11, 12, 13).   The contraction is found to occur in the double as well as in the single cones. The measurements of the double cones show that the myoid of the smaller member has shortened more than that of the larger (figs. 7 and 8).   Further evidence of changes in the length of the visual cells in light and darkness is afforded by a study of the relative positions of the cone and rod ellipsoids.   In the dark

condition (fig. 9) the ellipsoids of the single cones are usually found to be on the same level as the rod ellipsoids. On the other hand, in the light condition (fig. 10) the cone myoid is seen to occupy a level closer to the external limiting membrane than that of the rod ellipsoid. This change in relative position is the result of the combined effect of rod elongation and cone contraction,
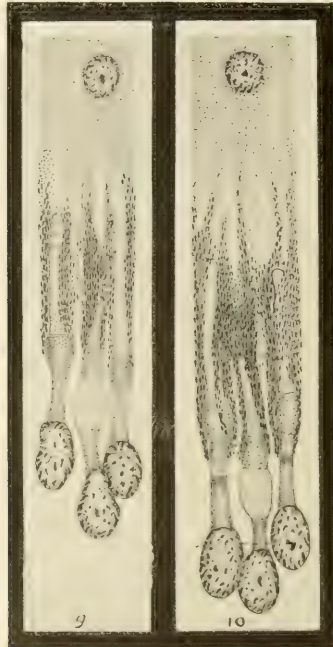
Fig. 9　A portion of the retina 3 mm. from the entrance of the optic nerve (region C). Animal in darkness for twenty-four hours. × 935.

Fig. 10　A portion of the retina from a region corresponding to that designated in figure 9. Animal in diffuse light for seven hours. × 935.

and is clearly shown by an examination of the rod and cone shown on the right-hand side of the diagrammatic figure 11.

Pigment migration. The differences in position of the pigment in light and dark eyes is slight. A series of measurements of the distance between the rod nuclei and the nearest pigment needle (table 1), as well as measurements of the distance between the

external limiting membrane and the nearest pigment needle, show that the pigment in the light eye is about 1.6μ nearer the external limiting membrane than in the dark eye. This almost insignificant amount, however, when combined with the distance the rod myoid has elongated in the light (4μ) gives an effective migration which is clearly illustrated in figures 9 and 10, as well as in figure 11. An examination of these figures shows that in the dark condition the choroidal portion only of the rod ellipsoid is covered with pigment, while in the light condition the entire
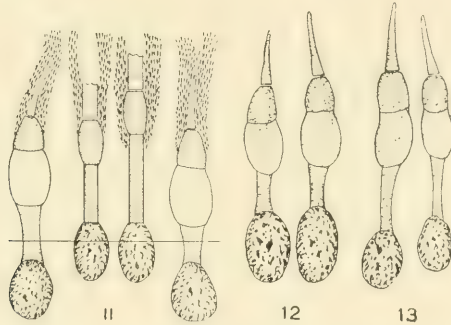
Fig. 11 Diagrammatic drawing compiled from 530 measurements showing the effects of light and darkness on the visual cells and on the position of the pigment. The left-hand side of the figure represents the dark condition; the right side, the light. The drawings were accurately laid out on coördinate paper from measurements presented in table A, each millimeter being given a value of 0.5μ.

Fig. 12 Single cones. Animal in diffuse light for seven hours. $\times$ 935.

Fig. 13 Single cones. Animal in darkness for twenty-four hours. $\times$ 935.

ellipsoid and a portion of the myoid is ensheathed. The amount of cone contraction in the light, being only slightly more than the extent of the pigment migration (table 1), the relation between the position of the pigment and the cone ellipsoid is practically the same in both dark and light eyes, the choroidal portion of the cone ellipsoid being in both covered by pigment (figs. 9, 10, and 11). The description of the position of the pigment in relation to the ellipsoids of the visual cells in both dark and light eyes pertains only to conditions found in the posterior part of the retina (about 2 to 3 mm. from the entrance of the optic nerve). Changes in the position of the pigment in

the more peripheral region of the retina could not be demonstrated. Near the margin of the retina the visual cells are greatly shortened and the pigment is found to extend down almost to the external limiting membrane.

DISCUSSION

Photomechanical changes of the retina. The question of the functional significance of pigment migration and the changes in position of the visual rods and cones in light and dark adaptation is one about which much has been written (Garten, '07, and Helmholtz, '11). It may therefore appear redundant to add anything in the way of a theoretical consideration of this function. But there are a few points which still lack clarity.

In the eyes of those animals in which these changes take place they represent a mechanism for the adaptation of the eye to day and to twilight vision (Herzog, '05; Exner and Januschke, '06). In dim light (twilight vision), when the rods alone are capable of being stimulated to any degree, or in complete darkness, the pigment moves back and leaves free the spaces between the rods, resulting in a less complete insulation of these elements. Under these conditions, with the entrance of a small amount of light the part played by the individual rods in the reception of the light is greater, owing to refraction and diffusion, than if the rods were covered over by a thick mantle of pigment, in which case only the light which passes through the retina in the direction of the long axis of the rods could enter them. The presence of a reflecting tapetum further enhances the favorable conditions. The cones in twilight vision are not functional, on account of their high threshold, and they elongate and thus move out of the way. The rods contract and thereby optimum conditions are presented for their stimulation.

In bright light (day vision) the pigment by migrating forward protects the rods, which have a low threshold and which have been made particularly sensitive by the accumulation of visual purple in the dark, from too strong stimulation by absorbing the direct and scattered light. The rods elongate, while the less sensitive cones are drawn out of the pigment by the con-

traction of their myoids and are thereby made freely accessible to the stronger light stimulus, thus presenting optimum conditions for their stimulation.

The fact that these photomechanical changes have not been demonstrated in the eyes of man and mammals does not constitute a denial of their taking place in the eyes of other animals, and there is no ground against explaining as above the phenomena in such animals. Adaptation of the mammalian eye is brought about by different means, viz., the formation and bleaching of visual purple.

The process of light and dark adaptation is not dependent upon the phototropic movements of the elements of the retina, but these movements may take part in the process of adaptation, that is in the formation and bleaching of visual purple. That the pigment, as such, has anything to do with the formation of visual purple is not probable, because visual purple occurs in the eyes of albinos and in the pigment-free portions of the retina of many animals, for example, the cat. The significance of the pigment is probably a purely optical one concerned with the absorption of scattered light.

In connection with the function of the retinal epithelium in the formation of visual purple, the paper by Kolmer ('09) is to be noted. Kolmer finds numerous droplets and granules on and between the rods in the retina of various vertebrates. These he regards as secretion products of the pigment epithelium. In the retina of frogs kept in darkness the droplets and granules are larger and more numerous than in the illuminated retina, and after illumination of the eye with direct sunlight are not to be seen at all. Since they are lacking in the eye of lizards and snakes, Kolmer assumes that they have something to do with the visual functions of the rods, the organs of twilight vision, and perhaps with the appearance of visual purple.

It is interesting to note that pigment migration is still assumed by some to take place in the human eye. Ramon y Cajal ('11, p. 363) believes that the function of the pigment is to prevent the impression of halo, and that the dazzling sensation which one experiences on going from a dimly lighted place into a bright one

is due to the fact that the pigment, which has moved back into the body of the epithelial cells as an effect of darkness, requires some time to be brought out again into the prolongations of the cells to ensheath the individual visual elements.

Bard ('19), in a highly theoretical paper, in many respects offering views widely divergent from those usually held concerning vision of form and of color, explains many things by the assumption that both pigment migration and cone contraction take place in the human eye.

Cobb ('19, p. 444) also states that pigment migration takes place in the human retina.   He says:

aside from the changes in the size of the pupil there are two anatomical factors undoubtedly concerned in dark and bright adaptation: the exhaustion and regeneration of visual purple (or possibly other photochemical substance); and the migration of the pigment of the hexagonal cells. This last may be a protecting device that acts fairly promptly, and has the effect of enclosing the retinal rods, and by its own light-absorbing qualities reducing the amount of light absorbed by the individual rods. It is conceivable that a sudden flash of light might anticipate this action and produce a strong destruction of the photochemical material in a short time, before the pigment cells have had time to react, while with gradual onset of light the time is adequate for the pigment cells effectively to assume this protective function.

And on page 445:

Some of the curves strongly suggest two factors playing a part in dark adaptation . . . . allowing the interpretation that the results are arising from two more or less independent mechanisms one of which overtakes the other in effect, at the end of about four minutes.

We believe that in the migration of pigment, the contraction of the cones, and the elongation of the rods there is exemplified the response of irritable protoplasm to a definite, adequate stimulus. In some cases the response is very marked, though of varying degree (fish, frog, bird); in others it is not demonstrable (man and mammals). In some it may serve an easily comprehended purpose; in others, in terms of the theory explaining it, it may seem to be useless. Nevertheless, if it can be demonstrated (as in the turtle, lizard, and alligator) it cannot be explained away or ignored because it seems to serve no useful purpose.

In the eye of the alligator the migration of the pigment and the change in position of the visual cells seem to be correlated with the relative distribution of rods and cones. The rods show the greatest difference in position in light and dark eyes, in the regions designated by C in figure 1 (rod-cone ratio 60 to 40), and by D (where the rods and cones are about equal in number), much less in the region B (rod-cone ratio 85 to 15), and not demonstrably at all in the region designated by A, or in the region (E and F) below the optic nerve, where the rods represent only about 5 per cent of the total number of visual cells. The pigment can be demonstrated to move forward only in the posterior portion of the retina (regions C and D), thus corresponding to the regions where the maximum change in position of the rods takes place. The cones throughout the retina show the same (slight) degree of shortening in the light, except that the double cones, which are most numerous in the regions B and C, show a slightly greater amount of change in length.

Garten ('07, p. 38) weakened the general application of the suggestion put forth by Herzog ('05) and Exner and Januschke ('06) by observations which seemed to show an extremely high sensitivity to stimulation by weak light, so that the light condition of the visual cells was considered as assumed in dim light. Arey ('19) has recently brought forward evidence indicating that the sensitivity of the retinal pigment and of the rods and cones is nowhere nearly so high as is generally believed, and the conception that the changes observed in those eyes where marked effects of light are obtained are adaptive has been thereby placed on much surer ground.

We should not, however, a priori, deny that light effects similar, if less marked, changes in retinae so constituted that there can be no, or little, question of any advantage to be gained by a correlative shifting of the position of the visual elements. Garten cites the facts that in the selachians, which presumably have pure-rod retinae, although the literature on the subject is not without disagreement,[1] there is little, if any, pigment, and that

[1] Schultze, '66; Krause, '76 and '95; Neumayer, '97; Schaper, '99; Hesse, '04; Franz, '05; Retzius, '05; Garten, '07; Cajal, '11, and Pütter, '12.

in pure-cone retinae, where the pigment, as assumed, is necessary for the absorption of the light scattered by the highly refractive cones, there is practically no pigment migration. But Detwiler ('16) found a demonstrable pigment migration and cone contraction in the eyes of both turtles and lizards. Garten further argues (p. 109) that photomechanical changes should not take place in the crocodilian eye, because of the exclusive presence of rods or cones in the various portions of the retina. But, as we have demonstrated above, the structural conditions, at least in Alligator mississippiensis, differ from his description, and changes in the position of pigment and of visual cells do take place in light and darkness.

The duplicity theory. The duplicity theory of von Kries, or the theory of the double retina of Parinaud, based on the findings of Max Schultze ('66), is of the greatest importance in comparative work on vision. The hypothesis is generally regarded as well substantiated, particularly by the facts of twilight and day vision. For brief accounts and references to the literature of the theory and its development the reader is referred to Nagel ('05), Helmholtz ('11), and Parsons ('15). Briefly stated, the theory holds that the rods are sensitive only to light and darkness, and by virtue of their power of adaptation in the dark through the regeneration of visual purple they form the apparatus for vision in dim light. The cones, on the other hand, are the apparatus subserving bright vision as well as the perception of color. But in another way, the rods are the apparatus for achromatic scotopic vision (twilight vision), the cones the apparatus for photopic vision (day vision). The cones are not necessarily assumed to be utterly useless at night, but only relatively so, being quickly fatigued, on account of their high threshold.

The presence and relative distribution of rods and cones is therefore a matter of the first importance. But without prejudice it can be said that this is a very unsatisfactory matter as far as the comparative literature is concerned. Early contributions to the histology of the visual neuro-epithelium either have not been reinvestigated, but assumed to be correct, or

when investigated a divergence in the opinions of later investigators is the rule rather than the exception.

The question as to what constitutes a rod and what a cone would seem to be a simple matter, but the great variety in the forms of the cells in different animals makes difficult a generalized classification. The contentions of Pütter ('12) for a functional classification based on the type of connection of the visual cell with the bipolar, rather than a structural one, are good if kept within limits, but it seems to us that they are carried too far. As everyone knows, the foveal cones of man and mammals are cylindrical in shape, and are therefore much more like rods in general appearance than cones. But their known function fits in with the general conception of the physiology of the apparatus for color and bright-light vision. Troland ('17) has pointed out that the shape of the foveal cones suggests that the function of the cone figure is structural rigidity rather than differentiation of response.

Whether a visual cell is a rod or a cone is determined by the presence of one or more of three structural factors, viz., 1) the shape of the outer segment; 2) the shape of the inner segment, and, 3) the mode of connection between the visual cell and the bipolar cell. When we find visual cells which, from their general form (outer and inner segments), would be called rods, possessing terminal connections typical of cones (e.g., frogs, diurnal birds, see Ramon y Cajal, '94, pp. 31, 164, and '11, pp. 340, 327), there is no, or very little, reason why they should be called cones simply because they terminate in dendrites, and considerable reason for continuing to designate them as rods on functional as well as structrual grounds. The conditions in the geckos may be cited as another example. From description and illustration it would seem as if no more typical rods could be found. Coupled with their structure there are functional characteristics, which will be referred to later, and which, from all that we know about rod vision, indicate that the visual cells are as functionally typical rods as can be found. Our work on the retina of the alligator shows that rods as well as cones occur there, structurally as well as functionally, as exemplified in the inverse changes in

light and darkness. But Pütter would call them all cones because their centripetal termination is similar to that found in the cones of man.

The designating a visual cell as a rod or as a cone on morphological grounds is not therefore useless, as Pütter claims, but, as he also points out the structural basis (form of inner and outer segments), is brought into line with the functional by what we know of the respective functions of the visual cells in man, viz., threshold, visual acuity, ability to see movement, and vision of color. The rods are visual elements with a low threshold, but with possibilities of summated conduction, due to the connection of more than one of them with a single bipolar cell; the cones are visual elements with a high threshold and isolated conduction, based on the histologically found type of connection.

Pütter, in speaking of the conditions in the nocturnal birds, admits that the visual elements have knob-like endings, and that the visual cells are morphologically typical cones, although they have assumed what he regards as the most distinctive characteristic of rods. Pütter reverses himself here and is, as well, incomplete, because, as Ramon y Cajal ('94, p. 104) points out, in these retinae there are rods ending like those of mammals, while the cones which have almost entirely similar endings, reach deeper and come into connection with a different set of bipolars, so that there is a further morphological differentiation here between rods and cones.

It does not seem at all certain to us that Hess ('10 and '13), by his work on the turtle and on birds, has disproved or weakened the general truth of the duplicity theory. He claims to have demonstrated an adaptation in the turtle retina, where there are cones only. The claim that he and Katz and Révész (13) make, that adaptation in diurnal birds is a function of the cones, does not seem warranted, owing to the fact that rods are present in considerable numbers, as Hess himself describes, particularly in connection with the presence of visual purple. The phenomenon, similar to the Purkinje phenomenon, which they state to have observed, may therefore, and most naturally, be a function of the rods and not of the cones. Katz and Révész

('13) also state that the rods of nocturnal birds (owls) in bright light approach, or are similar, in function to cones. But this is without anatomical foundation for the simple reason that the retinae of such birds contain numerous cones (Garten, '07; Hess, '13, p. 581) which show, with the pigment, photomechanical changes. In this connection the view of Parsons ('15, p. 204) may be quoted:

If we regard the rods as the more primitive type of visual neuro-epithelium, as we are probably justified in doing, the persistence of recognizable rod attributes in the cones, even if modified, differentiated, and rendered more complex, might well be expected. Apart therefore from the difficulties of isolating the physiological results of excitation of the rods from those of excitation of the cones it may be anticipated that the latter cells will retain some measure of the functions which are in the highest degree characteristic of their prototypes. Hence, if it should ever be conclusively proved that the rod-like foveal cones of the human eye possess some trace of visual purple and are endowed with some slight degree of light-adaptation it would not be surprising; neither, on the other hand, would it militate seriously against the view that the rods and cones have become essentially diverse in function.

Troland ('16) has demonstrated by careful experiments, corroborating the earlier work of v. Kries and Nagel, that the phenomenon of Purkinje does not take place in the rod-free portion of the human retina. And Watson ('15) and Lashley ('16) show that Hess' contention that the spectrum is shortened for the bird's eye as compared with the range of wave lengths seen by the human eye, is not correct.

It is not out of place to add that in the condition known as night-blindness, in which the rods are insensitive, or practically so, dark adaptation is almost abolished or is much slower than normal, and that Purkinje's phenomenon is much less marked than in the normal eye or absent altogether.

One further remark concerning Hess' work. He ('10) claims that many turtles are nocturnal and cites authorities supporting his contention. Ramon y Cajal ('11, p. 361) says that reptiles in general (in which of course he is incorrect, witness the alligator and the gecko) do not see in darkness. Rochon-Duvigneaud ('17) does not believe that turtles can be called nocturnal because they are incapable of flight from an enemy or of pursuing

prey, and he thinks that they detect the plants and insects upon which they feed by the sense of smell. Rochon-Duvigneaud asks the very apt question in reference to Hess' claim of adaptation in the fowl, why it is, if they possess the power of adaptation almost as well-developed as that of man, that they go to roost long before man ceases to enjoy good vision.

With reference to the duplicity theory and the distribution of rods and cones, the work of Abney ('16 and '17) is most interesting and important. By determining the minimum intensity of light of various wave length which can be perceived at the fovea and up to ten degrees from its center Abney and Watson ('16) obtained results indicating that in some cases the fovea of man is free from rods, which increase rapidly as the fovea is left, while in others there is a plentiful supply of rods at the fovea, their distribution, at any rate up to ten degrees, being very nearly uniform, and, if anything, in excess at the fovea. In the first group the light appears colored as long as it is visible at all, particularly in the green. In the second group the light loses color a considerable time before it is extinguished, except in the red. In other words, there is an achromatic interval. Abney ('17) later examined persons suffering from night-blindness, in which the rods are generally believed to be non-functional, for extinction of color from the red to the blue. The light was found to vanish when its color was extinguished, so that the same reduction in intensity of the light was the threshold for both light and color, similarly to the cases mentioned above where there are no rods at the fovea, indicating that there is an absence of sensitive rods in the whole retina of the night-blind.

If dark adaptation is directly associated with visual purple the vision of an animal possessing rods only as compared with that of an animal with cones only, both in respect to ability to see in light and darkness, after longer or shorter adaptation to the one or the other condition, and as well the relative stimulating value of spectral lights of equal energy, should be expected to be markedly different, quantitatively as well as qualitatively. Now we have in the reptiles admirable subjects for investigating this

very question, especially in the lizards, for example, the geckos as compared with other lizards and particularly the horned toad which has a retina, from all structural indications, peculiarly adapted for day vision only. By investigating in the gecko and the horned toad the relative visibility of wave lengths of equal energy and the relative powers of adaptation, we will obtain information concerning the question of the selective response of rods to different wave lengths as compared with that of cones. Visual purple absorbs all wave lengths except a little red and violet. The rods therefore are presumably sensitive to all wave lengths except the extreme red and violet. Since rods are in general 'color-blind,' there is opportunity here of differentiating between wave length and stimulating value.

In connection with the question as to which kind of visual cell represents the more primitive condition, the histogenesis of the retina is worthy of investigation. But the histogenesis of the neuro-epithelium is a subject about which our knowledge is most imperfect. In general, the rods are regarded as the more primitive type of visual cell (Graham Kerr, '19), while the cones are considered as specialized rods.

According to Leboucq ('09), the two kinds of visual elements develop simultaneously and are distinguishable only by the fact that the axis of the diplosome is perpendicular to the surface in the case of the rod and parallel to it in the case of the cone.

Cajal ('11, p. 356) says that the cones and rods evolve in the same way and that it is difficult to distinguish between them at the beginning (also Fürst, '04).

Cameron ('11) reiterates a view earlier championed by himself ('05) as well as by Bernard ('03) to the effect that the cones represent early stages in the formation of rods. We mention this here because of the fact that in looking over some slides of early amphibian embryos, the eyes do seem to contain nothing but cones or conically shaped elements. Graham Kerr (p. 137) finds that the visual elements (rods) in the retina of Lepidosiren shorten in the light and elongate in the dark, which is similar to to the usual behavior of cones and contrary to that of rods.

The shape of the pupil is a subject of interest in a paper dealing primarily with the eye of the alligator because of the vertical slit form of the pupil in this animal. In two recent articles reference is made to this subject. Rochon-Duvigneaud ('17), in listing the characteristics of the eye of the geckos which make them adapted to nocturnal vision, includes the form of the pupil—a vertical slit—which shows a rapidity of movement surpassing that of the human pupil and approaching that of birds. In dim light the pupil is a large oval, or even round, as in the cat. In bright light it is closed completely. According to Rochon-Duvigneaud, a round pupil can dilate as well as an oval one, but it cannot be entirely closed, and he believes that it is in the way of a protection against an excess of light in an animal adapted to twilight vision that an oval pupil finds its chief function. It is possible to imagine that a pupil in the form of a vertical slit can be opened wider than if it were round (for example, the cat).

Hartridge ('19) views the function of a vertical slit pupil (as seen in the cat) from another angle, viz., the function of the lens and the aberrations caused thereby, and the habits of life of the cat family in the nature of their being tree-climbing and tree-dwelling animals which hunt their prey chiefly at night. An oval pupil in which the long axis is vertical will cause the lens system to form images in which the aberrations of horizontal contours are greater than those belonging to vertical contours. The contours of trees and their branches are principally vertical, therefore if the illumination of the image formed on the retina could be increased by sacrificing the definition of horizontal contours it would be an advantage. This is effected by the use of the oval pupil since the aberration of vertical contours is little greater than that of a circular pupil of the same horizontal diameter, while the intensity of the image formed on the retina is as much the greater as the vertical diameter of the oval is greater than that of the circular pupil.

It seems more likely to us that the function of a vertical slit-shaped pupil is for protection; that is, to permit of its being almost, if not entirely, closed. In thinking of animals that have

this type of pupil, we find that many of them are essentially nocturnal in habits—the gecko, the alligator, the cat—animals in which the rod functions are predominant over those of the cones. At night or in dim light the pupil of the cat is wide open and round. Furthermore, in the daytime, when the pupil is a vertical slit or oval, cats hunt and catch a great deal on the ground, for example, birds and squirrels, as well as chase the rapidly swirling leaves. The cat is furthermore said to have very defective daylight vision and to be colorblind (DeVoss and Ganson, '15). The alligator hunts along horizontal contours, and yet one finds that the shape of the pupil is a vertical slit.

SUMMARY

1. The eye of the alligator possesses a well-developed retinal tapetum in the dorsal and posterior portions of the retina to within 1.5 mm. of the entrance of the optic nerve. It is formed by the inclusion of guanin in the cells of the epithelial layer (figs. 1, 2, 3, 4). The pecten consists of a slightly raised pigmented cap covering the entrance of the optic nerve.

2. Typical rods and cones occur throughout the retina, the cone-rod ratio being different for different regions, but characteristic for particular regions (p. 216).

3. The rods are all of one type (fig. 5), the cones of two, thick and thin, of which the first is by far the more numerous, occurring particularly in the posterior and ventral portions of the retina. Those of the second type are found only in the ventral portion and are not numerous (fig. 6). Double cones also occur (figs. 7 and 8). None of the cones contain oil drops. The rod nuclei are oval or elliptical in shape, and the majority of them project through the external limiting membrane for a variable extent, the rest of them being just under it. The cone nuclei are pear shaped and, occupying a deeper level than the rod nuclei, constitute a second row (figs. 5, 6, 9, 10).

4. The rods show a change in length of their myoids averaging 4μ, being longer in the light and shorter in the dark (figs. 9, 10, 11 and table 1). The single cones (thick and thin) show an average change of 2.1μ (figs. 11, 12, 13 and table 1). The

smaller member of the double cones shows an average change in length of 3.5μ, the larger member of 2.7μ (figs. 7, 8 and table 1).

5. The actual change in position of the pigment between light and dark eyes is slight, averaging but 1.6μ; but when combined with the change in length of the visual cells, gives an effective migration equal to the sum of the two (figs. 9, 10, 11).

6. A theoretical consideration of photomechanical changes and of the duplicity theory from a comparative point of view is appended.

BIBLIOGRAPHY

ABELSDORFF, G. 1898 Physiologische Beobachtungen am Auge der Krokodile. Arch. f. Anat. (u. Physiol.), S. 155–167.

ABNEY, SIR W. DE W., AND WATSON, W. 1916 The threshold of vision for different colored lights. Philosoph. Trans. Roy. Soc., vol. 216A, pp. 91–142.

ABNEY, SIR W. DE W. 1917 Two cases of congenital nightblindness. Proc. Roy. Soc., vol. 90B, pp. 69–74.

AREY, L. B. 1919 A retinal mechanism of efficient vision. Jour. Comp. Neur., vol. 30, pp. 343–354.

BARD, L. 1919 Du rôle des bâtonnets et des pigments rétiniens dans la perception des formes et des couleurs. Jrl. de Physiol. et de Path. gén., T. 18, p. 276–294.

BAYLISS, W. M. 1918 Principles of general physiology. Second edition. Longmans, Green & Co.

BERNARD, H. M. 1903 Studies in the retina. Q. J. Micro. Sci., N. S. vol. 46, pp. 25–75.

CAMERON, J. 1905 The development of the retina in amphibia. Jrl. Anat. and Physiol., vol. 39, pp. 135–153, 332–348, 471–488.
1911 Further researches on the rods and cones of vertebrate retinae. Jrl. Anat. and Physiol., vol. 46, pp. 45–53.

CHIEVITZ, J. H. 1889 Untersuchungen über die Area centralis Retinae. Arch. f. Anat. (u. Physiol.), Suppl. Bd., S. 139–196.

COBB, W. C. 1919 Dark-adaptation with especial reference to problems of night-flying. Psychol. Rev., vol. 26, pp. 428–453.

DETWILER, S. R. 1916 The effect of light on the retina of the tortoise and the lizard. Jour. Exp. Zoöl., vol. 20, pp. 165–191.

DEVOSS, J. C., AND GANSON, R. 1915 Color blindness in cats. Jour. Animal Behav., vol. 5, pp. 115–139.

EXNER, S., UND JANUSCHKE, H. 1906 Die Stäbchenwanderung im Auge von Abramis brama bei Lichtveränderungen. Ber. d. k. k. Akad. d. Wiss. zu Wien, Math.-Nat. Kl., Bd. 115, S. 269–280.

FRANZ, V. 1913 Sehorgan. Oppel's Lehrb. d. vergl. mikro. Anat. d. Wirbeltiere. G. Fischer.

Fürst   1904   Zur Kenntnis der Histogenese und des Wachstums der Retina. Lund's Universitets Arsskrift, Bd. 40, adfeln 1. No. 1 (cited from Cajal, '11).

Garten, S.   1907   Die Veränderungen der Netzhaut durch Licht. Graefe-Saemisch Handb. d. ges. Augenheilk. I. Teil, III. Bd., XII. Kap. Anhang., S. 1–130.

Hartridge, H.   1919   The shape of the pupil in various animals. Jour. Physiol., 53, vi.

Heinemann, C.   1877   Beiträge zur Anatomie der Retina. Ar. f. mikroskop. Anat., Bd. 14, S. 409–441.

Helmholtz, H. von   1911   Handbuch der physiologischen Optik. Dritte Aufl., Bd. 2. L. Voss.

Herzog, H.   1905   Experimentelle Untersuchungen zur Physiologie der Bewegungsvorgänge in der Netzhaut. Ar. f. (Anat. u.) Physiol., S. 413–464.

Hess, C.   1910   Untersuchungen über den Lichtsinn bei Reptilien und Amphibien. Pflüger's Archiv, Bd. 143, S. 255–295.
      1913   Gesichtssinn. Winterstein's Handb. d. vergl. Physiol., Bd. 3, S. 555–840. G. Fischer.

Hesse, R.   1904   Ueber den feineren Bau der Stäbchen und Zapfen einiger Wirbeltiere. Zool. Jahrb., Suppl. Bd. 7, S. 471-511.

Katz, D., und Révész, G.   1913   Ein Beitrag zur Kenntnis des Lichtsinns der Nachtvögel. Zeit. f. Sinnesphysiol., Bd. 48, S. 165–170.

Kerr, J. Graham   1919   Text-book of embryology, vol. 2, Macmillan & Co.

Kolmer, W.   1909   Ueber einen sekretartigen Bestandteil der Stäbchen-zapfenschicht. Pflüger's Archiv, Bd. 129, S. 35–45.

Krause, W.   1876   Die Nervenendigung in der Retina. Ar. f. mikroskop. Anat., Bd. 12, S. 742–790.
      1893   Die Retina der Reptilien. Intern. Monatschr. f. Anat. und Physiol., Bd. 10, S. 13–31, S. 33–62, S. 68–84.
      1895   Die Retina. Nachträge. Ibid., Bd. 12, S. 176–186.

Lashley, K. S.   1916   The color vision of birds. I. The spectrum of the domestic fowl. Jour. Ani. Behav., vol. 6, pp. 1–26.

Leboucq, G.   1909   Contribution a l'étude de l'histogénèse de la rétine chez les Mammifères. Arch. d'Anat. Microscop., T. 10, p. 555–605.

Nagel, W.   1905   Handbuch der Physiologie des Menschen, Bd. 3. Fr. Vieweg & Sohn.

Neumayer, L.   1897   Der feinere Bau der Salachier-Retina. Ar. f. mikroskop. Anat., Bd. 48, S. 83–110.

Parsons, J. H.   1915   An introduction to the study of colour vision. Putnam's Sons.

Pütter, A.   1912   Organologie des Auges. Wm. Engelmann.

Ramon y Cajal, S.   1894   Die Retina der Wirbelthiere. Bergmann.
      1911   Histologie du Système nerveux de l'homme et des Vertébrés. A. Maloine.

Retzius, G.   1905   Zur Kenntnis vom Bau der Selachier Retina. Biol. Unters. (N. F.), Bd. 12, S. 55–60.

Rochon-Duvigneaud, A.   1917   Les fonctions des cônes et des bâtonnets.   Indications fournies par la physiologie comparée.   Ann. d'oculistique, Nov., p. 16.

Schaper, A.   1899   Die nervösen Elemente der Selachier-Retina in Methylenblaupräparaten.   Festschr. z. 70. Geburtstag von C. von Kuppfer, S. 1–10.

Schultze, M.   1866   Zur Anatomie und Physiologie der Retina.   Ar. f. mikroskop. Anat., Bd. 2, S. 175–286.

Tafani, A.   1883   Parcours et terminaison du nerf optique dans la rétine des crocodiles (Champsia lucius).   Ar. ital de Biol., T. 4, p. 210–233.

Troland. L. T.   1916   Apparent brightness; its conditions and properties. Trans. Illum. Eng. Soc., Vol. 11, pp. 947–966.
1917   The nature of the visual receptor process.   Jour. Opt. Soc. of America, vol. 1, pp. 3–15.

Vincent, S. B.   1912   The mammalian eye.   Jour. Ani. Behav., vol. 2, pp. 249–255.

Watson, J. B.   1915   Studies on the spectral sensitivity of birds.   Carnegie Institute of Washington, Dept. of Marine Biol., no. 211, pp. 85–104.

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Las células germinales de los Anuros.

I. El ciclo sexual del macho de Rana catesbiana.

Las células germinales aparecen primeramente bajo la forma de una cresta mediana de células semejantes a las del endodermo, situado encima del techo del arquenterio, en los embriones de 7 mm. La cresta está separada del endodermo subyacente por: (1) La oclusión de las placas laterales y la formación del mesenterio, y (2) Por la emigración activa de las células germinales. La cresta germinal se divide longitudinalmente, y las mitades se separan para formar las crestas gonadiales pares de la larva. En la larva de la primera estación (de cuatro a seis meses de edad) las gonadas son simplemente sacos huecos cuyas paredes están formadas de una capa sencilla o doble de células sexuales. A pesar del carácter no diferenciado de las gonadas y de la falta de madurez de las larvas, las células sexuales pasan por un ciclo sexual muy precoz y abortivo, el cual termina con la degeneración y reabsorción de las células.

El fenómeno de la maduración es normal hasta la primera división de maduración, cuando la fragmentación del centrosoma, con formación consiguiente de poliasters, tiene lugar acompañada de la destrucción de los cromosomas. Se forman unas cuántas espermátidas gigantes mediante crecimiento de una fibra axial que crece del centrosoma de los espermatocitos primarios no divididos. Las células y los cromosomas se parecen mucho más a las de los Urodelos que a las células y cromosomas de los anuros adultos. Unas cuántas espermatogonias, descendientes lineares de las células derminales primordiales, persisten sin cambiar durante el ciclo sexual abortivo y producen una segunda generación de células germinales en las larvas de dos años de edad. Muchas de estas células pasan por un segundo ciclo de desarrollo y dan lugar a espermatozoides en el renacuajo. Por consiguiente, en la larva de la rana toro existen dos ciclos sexuales: El primero es muy precoz y abortivo, el segundo es normal. El autor interpreta este fenómeno como una recapitulación en el ciclo de las células germinales de condiciones filogénéticas que han pasado en los anuros.

THE GERM CELLS OF ANURANS

I. THE MALE SEXUAL CYCLE OF RANA CATESBEIANA LARVAE

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TWO TEXT FIGURES AND FIFTEEN PLATES (ONE HUNDRED AND THIRTY-ONE FIGURES)

CONTENTS

INTRODUCTION

Several years ago, while engaged in experimental work involving the germ glands and germ cells of anurans (Swingle, '17, also '17-'18), the writer was somewhat hampered by lack of definite criteria for differentiating the sexes in young larvae. In so far as the cytological conditions presented by the germ cells were concerned, it was impossible at that time, to distinguish clearly male from female tadpoles. The literature concerning sex in larval Anura was found to be voluminous and contained a great variety of opinions, many of which were mutually exclusive, others evidently based upon scanty evidence of somewhat dubious value, and none in any sense adequate to account for the conditions presented by my material. In the summer of 1917, therefore, an attempt was made to clear up the puzzling question of sex differentiation, but the effort proved abortive owing to lack of sufficient material. Certain cell stages occurred in my larval material which had been a source of mystification to the writer and to many others as well who had examined the material; these stages had apparently never been observed or at any rate reported by previous workers on anurans. Fortunately, an opportunity soon presented itself of working with Prof. E. G. Conklin, of Princeton University, who made a suggestion that further investigation has since shown to be correct, i.e., that I was dealing with a precocious maturation cycle in anuran larvae. Professor Conklin's suggestion throws an entirely new light upon the question of sex differentiation and development in the Anura, and brings the sexual conditions of these forms more nearly into line with those described for other vertebrates.

It is a pleasure to acknowledge my indebtedness to Professor Conklin for this illuminating suggestion, and for many others as well, which have made this work possible, for the time he has spent looking over material, and for the keen interest displayed in the progress of the work.

To Prof. N. P. Sherwood and Dr. Cora Downs, of the Department of Bacteriology of the University of Kansas, I am greatly indebted for aid in collecting 2000 tadpole specimens from the outlying districts of Douglas County, Kansas, during the summer

of 1918. To Professors Allen and W. R. B. Robertson, of the Department of Zoology, the University of Kansas, I am also indebted for aid in collecting tadpoles and newly metamorphosed bullfrogs at various times.

DIVISION OF THE PROBLEM

The subject of sex in larval anurans is such a complex one and the literature on the question so vast, that no attempt will be made to deal with all the aspects of the problem in this paper. Instead, the material has been so arranged that different phases will be taken up and discussed separately in a series of papers. This paper is concerned chiefly with the more usual phases of the sexual cycle of the male Rana catesbeiana, both in the larvae and newly metamophosed animals, with especial reference to chromosomal conditions. The broader questions of hermaphroditism, alleged to exist normally as a developmental phase of anurans, reversal of sexuality, anomalous sex ratios and their experimental modification, Bidder's organ, and other interesting problems will not be touched upon here, save perhaps incidentally, and then only in the briefest fashion. It will be recalled that Pflüger reported years ago, that there occur normally in newly metamorphosed frogs three kinds of individuals, males, females, and hermaphrodites, the two latter forms much more numerous in early stages than the males. In the course of further development the hermaphrodites become either definitely male or female, as the sex ratio for adult frogs is approximately 50–50. The investigations of R. Hertwig, Kuschakewitsch, and Witschi not only confirmed Pflüger's work, but extended it by showing that anurans apparently first develop solely as females and sexual intermediates, the males only later differentiating from the females and hermaphroditic forms. Moreover, these investigators described in great detail modification of the sex ratios by environmental changes, such as extremes of temperature and late fertilization. All of these alleged facts have given rise to the belief that anurans in their sexual development differ greatly from other vertebrates. These questions are reserved

for a later paper, which will be a consideration of the developmental history of the male and female sex glands, neoteny, Bidder's organ, and an attempt at a reinterpretation of the problems stated in the light of certain phenomena described below. The writer regards the second part of this work as perhaps the most interesting from a theoretical standpoint and as comprising the main portion; however, for sake of clarity in presentation, division of the subject has been found essential. It is necessary to give in detail the normal germ-cell cycle before discussing its aberrations or more unusual modifications.

MATERIAL AND METHODS

During the course of the work only one species of anuran has been employed to any extent, i.e., Rana catesbeiana. Other forms have been examined for comparison with the bullfrog, but not for the phase of the problem treated in this paper, so they need not concern us here. Rana catesbeiana in its larval stage has no equal among other frogs in respect to the peculiar fitness of its germ cells for this sort of study. The sex cells of the Urodela have long been noted for their size and fitness for cytological study, whereas the cells of adult frogs and toads have received scant attention. Yet it is a fact that the germ cells of larval bullfrogs, in regard to the size of cells and chromosomes, are little surpassed by even the best urodele material, and in this respect they more nearly resemble the caudate forms than the conditions presented by adults of their own species. In the adult frog or in newly metamorphosed animals the size of cells, nuclei, and chromosomes is distinctly less than in the larvae. The germ cells of sexually mature bullfrogs are in this respect like those of a different animal group when compared with larval stages. The explanation of this peculiarity will be discussed in its proper place.

Another interesting feature about the bullfrog that makes it an especially favorable object for study is its remarkable long larval life. This species usually spends several seasons as a larva, and is a tadpole for approximately two years. Sometimes these animals pass through almost three years as tadpoles, though this is

a rare condition and probably a result of defective thyroid development. The animals are abundant, are easily caught, and readily adapt themselves to laboratory conditions. Tadpoles caught in the autumn need not be fed more than once a month throughout the winter to keep them in good condition. First-season tadpoles rarely attain a greater length than 35 to 40 mm.; second-season specimens average 65 to 85 mm.; mature tadpoles, 100 to 154 mm. It is rare to find larvae with a greater length than 145 mm., though the writer recently caught two male specimens measuring 159 and 165 mm., respectively, from snout to tip of the tail; both had ripe spermatozoa in the gonads.

It will be shown later in this paper that the long larval life of Rana catesbeiana is correlated with a very interesting and suggestive phase of the germ-cell cycle—a phase which, while normally occurring in other anurans and probably in many other vertebrate forms, is brief, and apparently obscured by other developmental phenomena, hence not so easy of interpretation as the same condition in the bullfrog larva.

It should be stated here that there is apparently no seriation of germ-cell stages anteroposteriorly in the testis of larval or newly metamorphosed Rana catesbeiana such as has been described for various urodeles. The testis of a 40 to 50-mm. larvae is a narrow, flat, ribbon-like structure, gray-white in color, somewhat convoluted, attached by a mesentery to the inner edge of the ventral surface of the mesonephros. It bears little resemblance to the testis of the adult and is longer than the gonads of newly metamorphosed frogs. The relation of the glands of first-year animals to those of second-year larvae and newly metamorphosed frogs is indicated in text figure 1. The internal structure of these gonads is indicated in photographs (33 to 35, explanation of figures), where it will be readily seen that the center of the gonad consists of a large hollow (secondary genital cavity) surrounded by a germinal epithelium consisting of a single or double layer of germ cells in 40 to 50 mm. tadpoles and of many layers of cells in 80 to 90 mm. animals. In mature larvae and newly metamorphosed frogs the central cavity of the testis is obliterated at definite intervals by migration of

mesodermal cells from the mesentery and mesonephros (the so-called sex cords, a misnomer for they are in reality the anlagen of the rete or efferent apparatus).

Various fixatives have been employed, such as Flemming, Bouin's, Ezra Allen's ('16) modification of Bouin's fluid, and others. The best results were obtained with the last two fluids. The mesonephros was usually left attached to the testis. Sections were cut at a thickness of 8 to 10 μ. Even at this thickness it
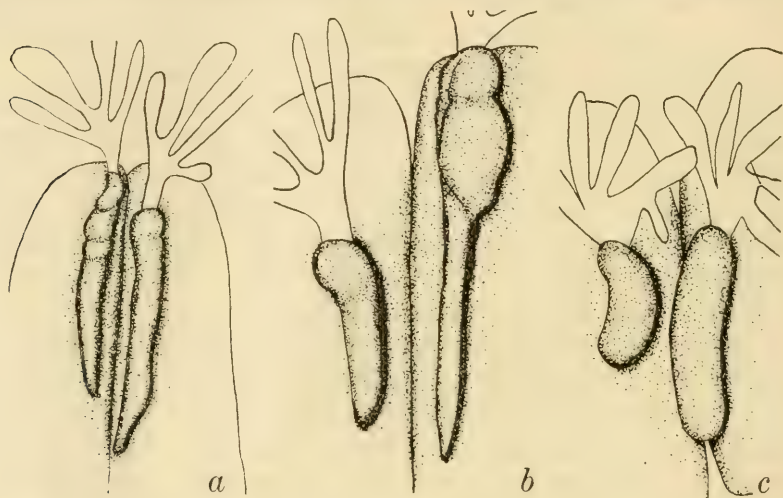
Fig. 1 *a*) Gonads of animal of first year. Average total length of larvae, 40 to 50 mm.; *b*) gonads of tadpoles 70 to 95 mm. total length; *c*) gonads of tadpoles nearing metamorphosis; total length, 120 to 150 mm.

is necessary to reconstruct the nucleus in most cases because of the large size of the cells. All spermatogonial counts, however, were made from complete cells.

The larvae used for the present work were taken from various localities and during different seasons of the year. Four hundred larvae measuring from 70 to 110 mm. were taken from the ponds of the State Fish Hatchery, Pratt, Kansas, during the month of September, 1917; 300 larvae averaging 100 mm. were taken from pools in the vicinity of Lawrence, Kansas, in the fall of 1916; a group of 1500 larvae averaging 70 mm. was caught in August,

1918, from a pool in Douglas County, Kansas; 1700 larvae measuring from 60 to 165 mm. total length were taken from a pond on the University Campus at Princeton during the months of July, August, and October, 1919. Only a comparatively small number of animals from these various groups were examined microscopically, the remainder were preserved for a study of the sex ratios and so-called hermaphroditism at various developmental stages—phases of the subject not dealt with here, but which make up the subject-matter of a later communication.

The size or length of tadpoles is not a good criterion of their age because of the size variability shown by anuran larvae of similar age, reared under identical environmental conditions. The writer was, until last year (1919), unable to get the eggs of the bullfrog in sufficient quantity to rear the tadpoles artificially. Hence the age of the older larvae given in this account is only approximate, for they are classified according to size and stage of development, as first- and second-year tadpoles.

RÉSUMÉ OF A FEW OF THE MORE IMPORTANT POINTS IN THE DEVELOPMENTAL HISTORY OF THE GERM GLANDS AND GERM CELLS OF RANA CATESBEIANA LARVAE

A brief summary of the developmental history of the gonads and sex cells may prove useful in elucidating some of the peculiarities of the sexual cycle described later in the paper. Only a few of the more important stages will be considered here, and then only in a very brief and sketchy way.

1. The primordial germ cells of the embryo are first distinguishable from other yolk-laden entoderm cells as a ridge just dorsal to the cavity of the archenteron and ventral to the aorta, separating the two lateral plates of mesoderm (text fig. 2, A). The medial growth of the two lateral plates and formation of the mesentery together with probably an active migration dorsally of the germ cells themselves, cuts off this germ-cell ridge from the underlying entoderm (text fig. 2, B and C). As development proceeds this median ridge of germ cells splits longitudinally and the cells of the two halves then migrate laterally on either side to form two independent ridges invested with a cov-

ering of peritoneum. In cross-section each ridge is seen to be
made up of several large yolk-laden germ cells and a few small
deeply staining peritoneal cells.

2. The two germ ridges enlarge considerably by proliferation
of the cells and also by migration of mesoderm cells into the
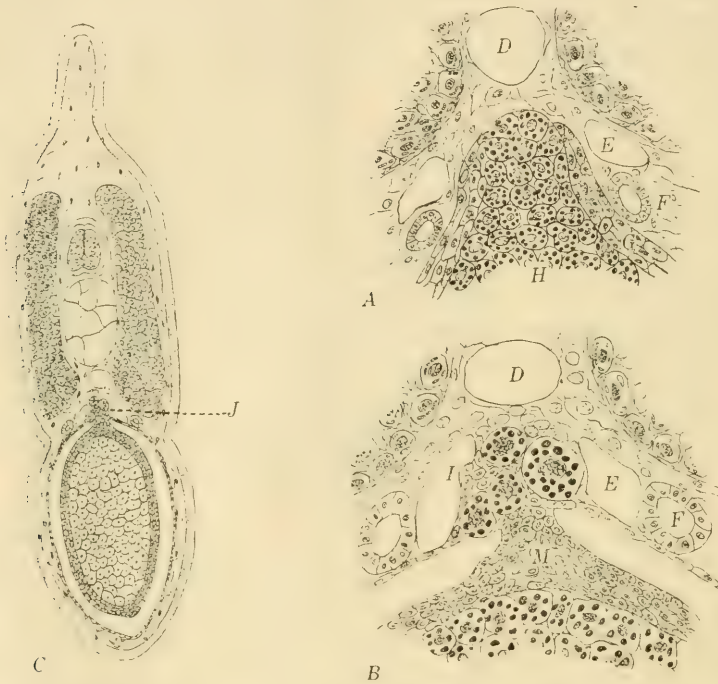
Fig. 2   Origin of the germ-cells. A. Cross section through germ-cell region
of 7-mm. larvae. D, the aorta; E, cardinal veins; F, wolffian duct; G, lateral
plate; H, entoderm cells. B. Transverse section through germ-cell region of
8-mm. larvae. I, germ-cells containing yolk; M, mesentery. C. Low magnifica-
tion of stage shown in B. J, germ cells.

ridges from the mesonephros and peritoneum. The ridges pro-
ject into the body cavity and take on the character of germ glands.
The germ cells lose their yolk at about this time and divide
actively.

3. As development progresses, the glands grow rapidly, the
number of germ cells greatly increasing. Large cavities are

formed in the gonads, the so-called secondary genital spaces, lined by small non-sexual cells which have migrated into the gland from the mesonephros by way of the mesentery. At this stage the gonads of both sexes are hollow sacs (surrounded by peritoneum, the so-called germinal epithelium), the walls of which consist of one, two, or three layers of sex cells, depending upon the stage studied (figs. 33 and 34).

In female larvae the cavity is later obliterated by growth of the oocytes. In male animals, these secondary genital spaces persist until shortly before metamorphosis, when they also are obliterated, chiefly by increased division of the germ cells, and the ingrowth of cells from the mesonephros, which form anastomosing cords throughout the testis, the future rete or efferent ducts (fig. 35 shows the obliteration of the testis cavity).

4. In young first-season tadpoles, the sexes are indistinguishable, though later males and females are easily separated by microscopic examination. The female glands grow very fast and greatly enlarge, owing to oocyte formation, becoming irregular in outline. On the other hand, the male gonads remain small, are fairly regular in outline, but do not generally assume the shape characteristic of the adult testis until some months previous to metamorphosis, i.e., until the larvae are about two years of age (fig. 35). Also text figure 1, C.

5. The germ cells of larvae, taken in summer of the second season, both male and female are found to be undergoing simultaneous maturation changes. This is a most unusual phenomenon, and so far as the writer is aware, unique among the vertebrates, though common enough perhaps among the invertebrates. In no other group of the Chordata has anything analogous to the simultaneous maturation changes of male and female germ cells of larval anurans, such as here described, been reported, although on certain theoretical grounds based on a study of the sexual cycle of the larval bullfrog, the writer ventures to suggest that analogous phenomena are likely to be found in the myxinoids, larval petromyzonts, and eels.

The early maturation stages preceding the growth period of the oocyte in female animals, such as leptotene, amphitene, pachy-

tene, and diplotene, are said to occur normally extremely early in most vertebrates, in some mammals before birth. In male individuals, according to the usual accounts in the literature, the same stages of the maturation cycle do not usually take place until shortly before the attainment of sexual maturity.

It has long been known that the germ cells of female frogs undergo the earlier maturation changes while the animal is still a tadpole. The growth period of the oocyte in this group of amphibia is assumed to last a very long time, though further work may show this not to be altogether true. According to several investigators of European frogs, the eggs are not ready for fertilization until the fourth or fifth season after metamorphosis, when the first polar body is extruded shortly before fertilization. In this connection Gatenby ('14) says of Rana temporaria:

Though one cannot be certain, I believe that an oöcyte takes two years at least, and more probably three to become mature. It is evident, therefore, that the young oöcytes formed in April or May in the adult will not be used for spawning next March, but certainly for a spawning several years ahead. The first ova derived from primordial germ cells would not be spawned till three years after the hatching of the tadpole, since the frogs around Oxford seem to become mature in three years.

However this may be, the germ cells of male Rana catesbeiana larvae enter maturation simultaneously with those of female tadpoles—long before metamorphosis, or before the gonads have even differentiated sufficiently to resemble a testis. Until the onset of maturation the gonads of the two sexes are morphologically identical. Following the precocious maturation cycle, it becomes easy to differentiate the sexes, as the female germ cells soon enter the growth period and become oocytes. The gross appearance of the glands of the two sexes changes at this time. The male germ cells pass through all stages of maturation, leptotene, amphitene (synapsis), pachytene, diplotene, tetrad formation, up to the first maturation division in a perfectly normal manner. During the anaphase of the heterotypic mitosis, or at the earlier period of spindle formation, the spermatocytes

undergo degeneration owing to fragmentation of the centrosome and consequent formation of polyasters which lead to aberrant divisions. Practically all of the first generation of male germ cells, i.e., those derived from the primordial germ cells of the entoderm ridge, pass through this abortive larval sexual cycle and degenerate in the act of division. A few of these cells give rise by direct transformation, without the intercalation of the first or second maturation division, to gigantic spermatids with axial filaments. Such spermatids possess fourteen tetrads. A very few of the primordial germ cells fail to pass through the precocious maturation cycle, and probably persist unchanged, apparently giving rise later by repeated division to a second generation of germ cells in the male. It may be remarked here that many cells of this second generation look as if they take origin from germinal epithelium elements, i.e., appear to be transformed mesothelial cells. This point, however, is still under investigation as morphological methods are not sufficient to determine whether or not such transformations actually occur. The mode of origin of the definitive germ cells of the adult is not strictly germane to this particular paper, and the question must be left undecided, pending results of experimental investigation.

This second generation of male sex cells, and this is the important point here, no matter whether they be lineal descendants of the primordial germ cells or transformed mesothelial elements, undergo a second maturation (sexual) cycle in larvae just ready for metamorphosis, i.e., in second-year tadpoles, and this generation of cells, oddly enough, gives rise to normal sex products, spermatids, and spermatozoa. The maturation cycle is normal in every respect. From the time of metamorphosis on to sexual maturity the young male frog apparently ripens his sex products continuously—this despite the fact that for a year or so, owing to his small size contrasted with that of mature females, he is probably unable to copulate.

It will be recalled that in the female sexual cycle the stage corresponding to the first spermatocyte division of the male is the stage of polar body formation which occurs normally at the time of copulation, presumably several years after metamorphosis.

Just why there should be this difference in time of maturation
between the male and female sexual cycles of the tadpoles the
writer is unable to say, though from certain data to be consid-
ered hereafter, obtained from studies on birds and mammals, it
would not be surprising if the young female frog some months
after metamorphosis likewise showed an abortive maturation
cycle culminating in degeneration of the oocytes.[1]   This point is
now under investigation.   Bearing in mind, then, this outline
sketch of the developmental history of the gonads and germ cells
of both sexes in the bullfrog tadpole, the following detailed
account of the cellular changes involved in the larval maturation
cycle of the male becomes more intelligible.[1]

OBSERVATIONS. SEXUAL CYCLE FIRST-YEAR LARVAE. PRIMARY AND SECONDARY SPERMATOGONIA

The primary spermatogonia found in such gonads as shown in
figures 33 and 34 and text figures 1 A and B are much larger
·than the later generation of cells to which they give rise.   In
general these primary cells are more lightly staining than other
elements of the gonad, and are peculiar, moreover, in that they
are usually surrounded by a follicle made up of small, flattened,
deeply staining stroma or peritoneal elements separating them
one from another.   This is true of this generation of cells in both
larval and adult frogs.

The primary spermatogonial nuclei are large and very irregu-
lar in outline, presenting marked lobulations and indentations—
the so-called polymorphism of the nucleus.   Study of these poly-
morphic nuclei during early prophase stages of division has led to
the conclusion that the lobulations and consequent polymorphism
are due merely to large chromosomal vesicles or to the partial
fusion of such vesicles, for from each of these lobulations a
chromosome or pair of chromosomes appear in division pro-
phases.   The resting nucleus contains considerable karyolymph,

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[1] Recently the writer has observed typical tetrad formation and a few first-
maturation spindles and chromosomes in oocytes of female larvae.   Such cells
degenerate in the act of division just as do the laval spermatocytes of the male
tadpole of the first year.

and an irregular linin network upon which is scattered chromatin granules of various size and shape, together with one or more nucleoli. The nuclear size is in many instances enormous, completely filling the cytoplasm except for a narrow peripheral border (figs. 1, 3, 115 and 117).

The character of the attraction sphere and centrosome presents nothing unusual and conforms to the type described for amphibians by earlier workers, hence it need not detain us here.

Division of the primary spermatogonia is always mitotic, and amitosis, though described for this type of cell in amphibians by La Valette St. George ('85), Meves ('91), Benda ('93), and McGregor ('99), has not been observed in Rana catesbeiana.

The somatic or diploid number of chromosomes in the male bullfrog larva is twenty-eight, and presumably this number is characteristic of the adult also, though no counts have been made on metamorphosed animals. A few years ago the writer found that twenty-six is the male diploid number for Rana pipiens, the leopard frog (Swingle, '17). Parmenter ('20) has recently confirmed this count for parthenogenetic frogs of the same species. According to King ('07), the somatic number in Bufo is twenty-four. This last number has also generally been regarded as characteristic for urodeles, such as Triton and Salamandra. Recently Snook and Long ('14) described twenty-eight chromosomes in the urodele Aneides lugubris, and Parmenter ('20) finds the same number in the larva of Ambystoma tigrinum. Levy ('14–'15) states that the diploid number in male Rana temporaria is twenty-five. The writer does not regard Levy's evidence as above criticism, and is much inclined to consider this statement as possibly a mistake. It would be odd if the males of all other amphibians, both urodeles and anurans so far studied, possessed an even number of chromosomes, and one species, Rana temporaria, possessed an odd number. Levy regards this species as having an accessory chromosome.

The writer described an odd chromosomal body in the germ cells of Rana pipiens as an accessory chromosome (Swingle, '17), but has since been in doubt in regard to this matter. The body described by me is probably a precociously dividing chromosome,

one-half of which sometimes migrates toward the pole of the spindle more quickly than does the other half to the opposite pole. The figures of Levy indicate that the body described by him as the sex chromosome is in all probability of the same nature as the precociously dividing chromosome described by myself. Further work on Rana temporaria will in all likelihood bring it into line with other species in regard to chromosomal constitution.

The twenty-eight somatic chromosomes of Rana catesbeiana may be divided into four groups: 1) Large V- or J-shaped elements; 2) intermediate sized J's; 3) small J's and, 4) slightly curved rods. These chromosomes appear to be definitely paired according to size and shape, and in this respect resemble those of other amphibians. It should be stated, however, that the chromosomes, though occurring in pairs in regard to size and shape relations, are not always found side by side within the nucleus. Many times the members of a pair are widely separated and may be on opposite sides of the nucleus. In general, though, the two homologues are usually near one another. Certainly, the intimate pairing of somatic chromosomes, such as described by Metz ('14) for Drosophilia and by Whiting ('17) for the mosquito, does not occur in the Anura (figs. 4 to 6).

The size and shape of the spermatogonial chromosomes vary somewhat with the fixative used, particularly if the fixation is not of the very best. The size variation is due to the preserving fluid and not to any real variation of chromosomal size or shape in the living tissue. In extreme cases the chromosomes may appear as short blocks, and their characteristic shape is entirely lacking (fig. 5). It is interesting in this connection to compare King's ('07) figure 10, plate 1, with my figure 5. King regards the chromosomes figured by her as those by young spermatocytes before the stage of reduction (p. 368). They look very much like the short dumpy chromatin blocks of my figure 5. This cell is an ordinary spermatogonium in prophase, in which the spireme segments have either been greatly condensed by imperfect fixation or else the cell was abnormal, probably the latter is the case, as such cells appear in otherwise excellently

fixed material. The chromatin masses are readily counted and are of the diploid number. This type of cell is unusual in the larvae, and has never been observed in metamorphosed frogs.

In metaphase the apices of the J-chromosomes are oriented toward the center of the spindle, and spindle fiber attachment is non-terminal.

The spermatogonial chromosomes are occasionally split into two elements twisted about each other as apparently is the usual condition in Ambystoma (Parmenter, '20).

Variations in the chromosome number have been observed in but two cases: once in a spermatocyte which contained eighteen tetrads, possibly the result of fusion of two adjacent cells, and once in a spermatogonium containing thirty-six or more chromosomes (fig. 19).

It is doubtful if variation of chromosomal number occurs in normal cells within one and the same individual, save perhaps in those cases where a single chromosome may occasionally undergo fragmentation. Even in such cases there is apparently no real variation in quantity of chromatin mass. Such chromosomal fragmentation as is described by recent writers, notably Hance ('18), has not been observed in the bullfrog except in degenerating first spermatocytes where the multipolar spindles literally tear the chromosomes to pieces (fig. 115).

The multiplication of secondary spermatogonia in the larval gonad, and this is especially true of first-year tadpoles, does not continue long enough to obliterate the lumen of the gland or to crowd the cells together owing to greatly increased numbers. During the second season the spermatogonial divisions come to a close and maturation begins in the type of gonad shown in figures 33 and 34, also text figure 1, A.

Last spermatogonial division. First-year tadpoles

The telophases of the last larval spermatogonial divisions differ in no respect from other similar stages in the mitosis of the primary and secondary spermatogonia. The period of nuclear reconstruction, however, presents marked structural changes differentiating it from all previous stages, in that the nucleus enters

the so-called 'resting' period, preparatory to undergoing the complex phenomena of maturation. As stated before, the nuclei of the primary and secondary spermatogonia soon become polymorphic in character, following division, and the chromatin material is found scattered throughout the nucleus in bead-like masses or granules, attached to an irregular linin network. In sharp contrast to this type of nuclear reconstruction, nuclei of the last spermatogonial telophase are round or oval in shape and of small size. The chromatin is in the form of small lumps or blocks (Janssens, '03) somewhat irregular in outline. In especially favorable cells the number of these blocks can be made out with a fair degree of accuracy. Their number is certainly diploid. The writer is inclined to regard these chromatin blocks as representing individual chromosomes at this stage. In early stages they are independent of one another, but very soon anastomosing linin fibrils appear between them.

The preleptotene period (Grégoire, '07) marks the first indications of resolution of the blocks. They become woolly or mossy in appearance, delicate, much coiled, and tangled thread-like processes appear, as if spinning out from the chromatin material in the mass. During the course of these changes the nucleus increases in size (figs. 8 and 47). These tangled threads so characteristic of the preleptotene elongate, lose their spiral-like character, and extend across the nucleus in loops. At this period there is no definite orientation of the leptotene filaments. Apparently, for the writer cannot speak with certainty on this point, each of the chromatin blocks of the telophase nuclei gives origin to a single thread. It is difficult, if not impossible, to unravel the snarl of elongating threads crowding the nucleus at the time of their first appearance. Wenrich ('16) has been able to trace the origin of the leptotene threads with considerable clearness in Phrynotettix magnus, and he is of the opinion that a single chromatin block gives rise to a single filament. This view seems very probable, when consideration is taken of the fact that the number of leptotene filaments is diploid and corresponds closely, if not exactly, to the number of blocks. Judging from Wenrich's figures, conditions in Phrynotettix at this period are much more favorable for study than in Rana catesbeiana tadpoles.

Shortly after their formation the leptotene threads tend to show a definite orientation of their free ends toward the centrosome and sphere in many cells, giving the appearance of a series of delicate loops. Janssens ('05) has characterized this orientation as the bouquet grêle or leptotene bouquet. The chromatin portion of the looped threads is in the form of very minute particles distributed at more or less regular intervals along a central linin core or fibril. Usually at this stage one or more nucleoli are present, though they differ from ordinary nucleoli in being in intimate connection with the chromosomes. These bodies have been termed chromoplasts by Eisen ('00), who first studied them in Batracoseps. We shall have more to say about these bodies later (figs. 10 and 55).

Following the period of the leptotene bouquet, there occurs in amphibians an extremely important and interesting stage, first observed by Janssens ('05) and named by him amphitene. This stage marks the first formation of the thickened pachytene thread and corresponds to the zygotene of Grégoire's ('07) terminology. In Rana catesbeiana larvae the amphitene constitutes a very definite and well-marked period in the maturation process—one that is easily differentiated from the leptotene preceding or the pachytene following. Judging by descriptions of various investigators of the zygotene in different animal groups, the amphitene of the amphibian germ-cell cycle is a prolonged transition stage between leptotene and pachytene. In typical amphitene nuclei one finds the nucleus marked off into two more or less distinct portions by the type of chromatin thread present. At the proximal pole of the nucleus, i.e., that side nearest the centrosome and sphere, the delicate leptotene filaments have disappeared, and one finds only the thickened pachytene threads; conversely, at the distal pole of the nucleus, i.e., the pole opposite the sphere, the leptotene condition persists. By focusing through a single cell, it is possible to bring into view now a leptotene, now a pachytene condition (figs. 11 to 13, 36 to 38). The explanation of this apparently anomalous condition is simply that the thick pachytene threads of the proximal pole of the amphitene nucleus represent the longitudinal fusion (parasynapsis) of two originally distinct leptotene

filaments. The side-by-side fusion or synapsis begins at the ends of the threads nearest the centrosome, and extends distally until fusion is complete throughout the length of the conjugants. Thus the amphitene is essentially a transition period in which the pairing of chromosomes in the stage of leptotene filaments is progressing. In the distal portion of the nucleus, where typical leptotene conditions persist, parasynapsis has not yet occurred.

The evidence for this point of view is quite conclusive in Rana catesbeiana larvae: a) The leptotene threads are certainly nearer the somatic number than the haploid number; b) the thickened pachytene loops represent the haploid or reduced number; c) the thickness of the pachytene elements is just twice that of a single leptotene filament, d) and, perhaps most conclusive, it is not difficult in studying amphitene nuclei, to trace the two unpaired ends of the leptotene threads from the distal pole into a single thickened pachytene thread at the proximal pole (figs. 11 and 12). Janssens ('05) has figured this stage clearly in his figures 20, 21, 22, and 23, plate IV. Wilson ('12) observed the same thing in Batracoseps material obtained from Janssens, and states that the conditions described are even clearer than Janssens figured them. Apparently analogous conditions are figured by Wenrich ('16) (fig. 77) and designated by him as zygotene stages showing incomplete conjugation of chromosomes.

The leptotene threads appear to coil or twist about each other corkscrew fashion so tightly that all trace of their double nature is lost and the resulting thickened thread appears single. (Fig. 12.)

Many investigators of amphibian spermatogenesis have described other methods of synapsis for this group of vertebrates. However, the period assigned, which has usually been considered as identical with synapsis in urodeles and anurans, is in all probability an artifact due to imperfect fixation of material, poor staining, or both. This so-called synaptic period corresponds to what McClung has termed synizesis or the "unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte." Nuclear conditions are at this time extremely difficult to make out, to say nothing of interpreting correctly. The pachytene spireme is considered as evolving out of this contracted nuclear condition (King, '07, figs. 24 and 25).

Janssens ('01) first called attention to this condition in urodeles and considered it a definite stage of the germ-cell cycle. Later ('05) he reversed his earlier opinion and stated the condition described earlier was due to poor fixation.

In the bullfrog larva there can be no question that synizesis is an artifact due to poor penetration of fixatives. For instance, in well-preserved material it is impossible to find contraction stages; where large pieces of the gland are used, generally the peripheral portion of the tissue will show no synizesis, whereas the central portion will show numerous contraction figures. A comparative study of reagents, such as Bouin's or, better, Ezra Allen's modification of Bouin's fluid without urea which is a good fixative for frog material, with Flemming's osmic fixative, a rather poor penetrant, on similar sized pieces of gonad, gives illuminating results in regard to contraction stages. In Rana catesbeiana and Rana pipiens slow penetration of fixatives clumps the delicate loops of the leptotene bouquet into a typical synizesis figure.

The condition described here for anurans possibly is not comparable to a somewhat similar clumping of nuclear contents in other forms described by various investigators. The writer has had the opportunity, through the courtesy of Dr. E. L. Shaffer, to examine synizesis stages in Cicada material. The conditions presented by this form are hardly comparable to those described here for anurans, and it may well be that in certain groups synizesis is a definite stage in the maturation cycle.

The partial synapsis of leptotene threads in the amphitene is completed in the pachytene stage which immediately follows. The threads of this period are thickened throughout uniformly and usually show no trace of their dual nature, save perhaps in respect to size. It is odd that in a fully formed pachytene spireme there is usually no indication of the leptotene threads which entered into its formation (fig. 39, also 11). Most animals show distinct traces of a primary longitudinal split or line of fusion between the conjugants. For example, Wenrich ('16), describing the pachytene stage in Phrynotettix magnus, states: "The line of separation between the threads which have conjugated (i.e the primary longitudinal split) remains visible throughout the pacytene stage."

It is generally only in the amphitene and diplotene that the line of fusion of the conjugants is visible in the bullfrog larvae. In this connection it is interesting to note that Janssens ('05), in his study of Batracoseps, was unable to detect any indication of a paired condition. Wilson ('12), in his examination of the same animal, agrees with Janssens that the pachytene threads appear as if single.

The pachytene period in anurans larvae is in all respects like that described for urodeles. In many cases the free ends of the thickened threads are applied close to the nuclear membrane at the proximal pole, the broad loops extending distally, thus giving rise to the pachytene bouquet. Janssens ('05) divided the pachytene in Batracoseps into two distinct periods: the 'bouquet orienté,' corresponding to the condition just described, and the 'bouquet transverse,' in which the nuclear contents have apparently rotated in relation to the sphere, so that the bouquet instead of being oriented toward the centrosome and sphere is turned at right angles to it. The writer is unable to say definitely whether the period of the transverse bouquet does or does not represent a well-marked stage in the maturation cycle of Rana catesbeiana. Very probably this stage is more marked in urodeles than in anura.

In Bufo, King ('07) derives the pachytene spireme from the irregular, deeply staining, confused chromatin mass of the synizesis period. Her figure 25, plate 1, is a clear expression of her idea regarding the derivation of the pachytene threads. According to her account, it is a continuous spireme, does not show any evidence of longitudinal splitting, and later in the course of development segments transversely into the reduced (haploid) number of chromosomes. If this account of conditions in Bufo is correct, then this anuran differs from other amphibians, both caudate and tailess, in respect to formation of the pachytene spireme and the tetrads. The writer is under the impression that the difference between Bufo and other forms rests upon a misinterpretation of synizesis and synapsis, and if reexamined Bufo will very likely be found to conform to the amphibian type of maturation cycle.

Following the pachytene is the period of exconjugation or disjunction of the homologous chromosomes, i.e., the longitudinal splitting of the thick double threads into two thin threads which diverge in the center, but remain united at both ends (figs. 14, 15, and 40 to 51). This stage corresponds to the diplotene of Winiwarter ('00) or the prostrepsinema of Janssen ('05). The pachytene threads split longitudinally, the split first appearing apparently at the distal pole of the nucleus and extending proximally. The line of cleavage might possibly be looked upon as marking the earlier line of fusion of the two originally unpaired leptotene threads, and hence be regarded as the line of disjunction (figs. 14 and 15, also 40 to 51). This is only guesswork, however, because in general the fused leptotene threads show no sign of separation in the pachytene as they do in other forms; i.e., the primary longitudinal split is usually invisible at this stage.

The diplotene stage in Rana catesbeiana larvae is marked by extreme growth of the cell, especially the nucleus which reaches gigantic proportions in many instances. In general the cells of the pachytene stage, though larger than those of the leptotene, do not present such marked size differences over leptotene stages as do the diplotene nuclei over both pachytene and leptotene.

In early diplotene, when the primary longitudinal split is just making its appearance, there is somewhat superficial resemblance to the amphitene stage. The similarity is, however, slight, and one could hardly confuse the two periods. The longitudinal split of the diplotene appears first at what corresponds to the distal pole of the double thread. In the amphitene just the reverse condition is presented, the initial pairing of the leptotene filaments begins first at the proximal pole. The very obvious difference in the size of the nuclei of the two periods is an excellent criterion for distinguishing the two stages. Also separation is never complete in the diplotene, as the homologues remain united at their ends; conversely, in the amphitene the unpaired leptotene threads at the distal pole of the nucleus diverge widely from one another.

Shortly after the process of disjunction, a secondary longitudinal splitting of each member of the pair appears, forming the

tetrad-complex, made up of four chromatids (McClung, '00) united at the ends. This secondary split marks the line of separation of the chromatids in the subsequent equational or homotypic division, i.e., the second maturation division. In each chromosome this second split is apparently at right angles to the primary split. Coincident with the appearance of the secondary split is a process of shortening and thickening of the diffuse, thread-like tetrads. This shortening and condensation of the chromosomes marks the end of the diplotene (figs. 16 and 17).

Following the stage just described, there occurs a series of transition stages leading up to the complete formation of the heterotypic tetrads on the mitotic figure. These stages are known by various names, but for present purposes Häcker's ('95) term 'diakinesis' will be employed as including that period in the germ-cell cycle, from the first formation of the tetrads to their definitive arrangement upon the first maturation spindle.

Diakinesis—formation of the tetrads

The ring tetrads, so characteristic of the Amphibia, are formed by the disjunction of the homologous chromosomes that paired during the amphitene and pachytene and separated during the diplotene, except at their ends which remained in contact (figs. 14, 16, 41 to 51). Thus, in the writer's opinion, the annular space represents, in Rana catesbeiana, the space between homologous chromosomes. In other words the space between the rings represents the 'primary longitudinal split' and probably the original line of fusion in parasynapsis of the autosome pairs. The first maturation division in the bullfrog larva is heterotypic or reductional for most of the tetrads in the sense that entire chromosomes are separated. This conception has been held by various workers on urodele spermatogenesis. Thus Janssens holds this view for the urodele Batracoseps and the anuran Alytes and Montgomery for Plethodon cinereus and Desmognathus fuscus, though the latter writer arrived at this conclusion by assuming telosynapsis occurs first. He interpreted the pachytene loops correctly as bivalent chromosomes, but he misinterpreted the nature of the double spireme, in considering each loop as two

univalent chromosomes united telosynaptically at the angle of the loop. According to this point of view, the space between the two arms of the loop is the space between two univalent chromosomes, but does not represent the line of fusion of originally separated leptotene threads. This view, while erroneous (admitted to be so by Montgomery himself, '12), leads to essentially the same end results as those stated by the writer.

There are fourteen typical rings and crosses plainly of tetrad nature in the spermatocytes of the bullfrog larvae and in some cells a large rod-shaped body may appear (fig. 26 to 28). The rings are of large size as compared with similar chromosome stages of adult frog material and are practically identical with those of urodeles in regard to size and shape. In the larvae these rings can be grouped according to their size relations—one very large ring (fig. 18), five intermediate in size (fig. 20), and eight smaller ones (fig. 21). The size relations of these rings in various cells is apparently constant for the species in cells of the same size, and this is an important point, for there is a variation of chromosomal size in cells of different size. The amount of volume of cytoplasm has much to do with the size of the chromosomes. Figures 29, 30, 31, and 32 bring out clearly this difference in chromosome size when two cells in identical stages but of different size are compared. The thinness of the chromosome group in figure 78 is not entirely due to stretching on the spindle. The chromatin mass varies in proportion to cellular size. i.e., the larger the cell the larger the chromosomes, and vice versa. Conklin ('12) has clearly shown and discussed this point also in numerous other papers. The extremely large cells shown in plates 7 to 14 have relatively large tetrads, conversely the smaller cells figured in plate 15 have much smaller tetrads. The photographs of plates 7 to 14 are of larval cells, those of plate 15 of the germ cells of animals at the time of metamorphosis.

Ring tetrads in amphibia have been described by several previous writers, so only a brief discussion is needed here. Following the separation save at the synaptic ends in the diplotene of the paired elements, and the appearance of the secondary split, the rings open in two planes at right angles to each other: 1) In the

center probably along the original line of fusion of the homo-
logues; 2) along the line of the secondary split after the fashion
described by Robertson ('14) and Wenrich ('16) for the Orthop-
tera.   Condensation of the chromatin begins at this stage.   In
early stages of ring formation the tetrads stain rather lightly
and are somewhat (fig. 17) diffuse, but as condensation proceeds
they readily take up the basic dyes.   The tetrad character is
obvious from a study of the synaptic ends of the homologues.
The larger rings in middle prophase stages are generally in the
form of figure 8's, and this character may be maintained up to the
metaphase.   The smaller tetrads early assume the character of
the rings; other shapes, such as crosses and y's, and in some cells
a rod, appear.   There is usually a single Y and a single cross-
shaped tetrad in every spermatocyte, though these may appear
much like small rings.   Other shapes that appear are transitory
stages in ring formation, or else portions of rings viewed from
various angles.   Large crosses, for instance, sometimes appear
in early prophase, and are generally true rings viewed 'en face,'
the arms of the cross being the long synaptic ends of the paired
chromosomes.   Such crosses are not comparable to or to be con-
fused with true cross-shaped tetrads (figs. 19).

In sections slightly overstained, the smaller rings appear solid,
the synaptic ends being represented merely by rounded knobs.
At times such rings may even appear like dumbbells, and this is
notably true of the second-year spermatocytes, i.e., those that
give rise to true spermatozoa at the second ripening of the germ
cells of the larvae (figs. 120 to 128).   Indeed, it is not improbable
that those investigators of anuran spermatogenesis who have
described prophase tetrads as solid and of dumbbell shape were
perhaps dealing with either overstained or imperfectly fixed
material.

Spindle-fiber attachment is non-terminal usually, but may occur
anywhere in spermatocytes of the first-year larvae showing cen-
trosomal fragmentation.   There are no normal spermatocytes in
the first maturation cycle, so any discussion of spindle-fiber
attachment is useless (figs. 29, 31, and 32).

During late stages of diakinesis the cells in many cases become greatly enlarged and in many instances are of giant proportions (figs. 64, 67, 112). The increase in volume may affect either nucleus or cytoplasm or both. It is rather common in my material to find over one-half or two-thirds of a nucleus in a single section because of the size, and it may be added that my material was sectioned at a thickness of 8 to 10 μ. These large spermatocytes of the larvae resemble those of urodeles more than adult anurans. It is an interesting and suggestive fact that near the period of metamorphosis the elongated, more or less ribbon-like testis becomes transformed into a very small typically shaped frog testis. The shortening process may require a considerable time, though the writer is inclined to doubt this on account of the absence of transition stages. The shortening progresses from posterior to anterior and may amount to as much as 1 mm. Figure 35 is a section through gland from a newly metamorphosed animal; figure 34 a gland of the second season (before metamorphosis); figure 33 a section of a gland of a first season larva.

Examination of the small, fully formed testes (full formed except for the efferent or rete apparatus) of recently metamorphosed animals reveals some interesting size differences of the cells compared with those of the gonads of young larvae of the first season. The cellular elements of the small gonads are more nearly like those of the adult, and it is rare to find the giant spermatocytes of the type figured in plates 7 to 13. The primary spermatogonia are of about equal size with those of the younger tadpoles, the chief differences are in the spermatocytes and diplotene and pachytene nuclei. These small testes first appear in tadpoles measuring 120 mm. or more from snout to tip of tail. Such animals are about a year and a half old or perhaps somewhat younger and are due to metamorphose the following summer. The hind legs are on the average about 25 to 30 mm.; the fore limbs are not visible. Now, oddly enough, some of the male animals are mature, in so far as the possession of ripe spermatozoa is concerned. And, as we shall shortly see, this character marks off this type of gland from those of the first-season larvae. As was stated before, the bullfrog tadpole passes approximately two

years as a larva, and each year is marked by a seasonal ripening
of sexual products. Attempts of first-year tadpoles to ripen
their sex products is abortive; the second year's attempt is suc-
cessful (plate 15) at the time of metamorphosis.

The heterotypic mitosis of first-year larval spermatocytes

First maturation division metaphases are very abundant in
first-year larvae, especially in young larvae 45 to 60 mm. total
length which have passed through one winter as larvae. In such
animals entire cysts are found with completely formed tetrads
and spindles all ready for division, and many in the act of divid-
ing; yet, oddly enough, careful examination of many hundreds of
sections of such larval gonads fails to show stages of the first
maturation mitosis beyond very early anaphases (plates 9, 10,
11). This is an interesting fact, and it is strange to see entire
cysts of apparently normal spermatocytes in metaphase or early
anaphase, and yet never find telophases of such divisions, inter-
kinesis stages, or any indications of secondary spermatocytes.
Cells which have developed thus far in a perfectly normal man-
ner, save for precocity of the maturation cycle and are apparently
in possession of the requisite mechanism for cell division, are
unable to complete the process. During late prophase and meta-
phase the achromatic elements, that is, the machinery of cell divi-
sion and chromosomal separation, break down and the tetrads go
to pieces before the telophase. It is rare to find complete
separation of the homologous components of the tetrads. Some-
times the smaller ring elements do separate (figs. 29 and 30) and
in rare cases an early anaphase is reached. In general the degen-
erative processes set in shortly after the time of spindle formation
when the chromosomes are arranged in a typical metaphase plate,
their long axis parallel to the long axis of the spindle, or else
when they are scattered irregularly through the cytoplasm, fol-
lowing the disappearance of the nuclear wall (figs. 100 to 110).
The spermatocytes may even go to pieces in late diakinesis,
though such cases are not common.

The obvious cause of degeneration of the spermatocytes of first-season larvae is to be found in the abnormal behavior of the centrosomes. Very early in the work it was observed that multi-polar mitotic figures were exceedingly frequent, and, indeed, these came to be regarded as the rule rather than the exception in the maturation cycle of first-year tadpoles. Triasters and tetrasters with striking and bizarre chromosomal arrangement proved so common that attention was focused upon the centro-some as the primary seat of degenerative processes. It may be added here that these aberrant polyasters are very favorable objects of study in Rana catesbeiana larvae because of their size and number and should prove of interest to anyone concerned with cellular mechanics.

Fragmentation of the centrosome of first-year larval spermatocytes

A study of the centrosome of the first-season spermatocytes proved very fruitful in several respects: 1) it gave the clue to correct interpretation of the anomalous behavior, i.e., the failure to divide of the spermatocytes; 2) the results of such study explained on sound mechanical grounds the presence of the poly-asters; 3) it led to the discovery of certain giant spermatid-like structures.

In most, if not all, of the first-season larval spermatocytes, the centrosome behaves abnormally, rarely does it pass through the normal cycle and give rise to a typical bipolar spindle. The usual thing is fragmentation of one or both halves of the divided centrosome; figures 18, 21, 79 show such fragmentation. There may be a central granule, surrounded by four or five others, all connected to one another by very delicate filaments. Each of these granules may or may not form a tiny aster in the cytoplasm. In other cells a typical spindle may be formed at one pole with numerous smaller spindles at the other pole. There are several variations of this type (figs. 18, 21, 79).

Perhaps the most peculiar condition noted in the centrosomal behavior of the spermatocytes was the tendency to form axial filaments or tails. It required much searching to find anything

like 'tailed cells,' and in the writer's experience they occur rather
infrequently, although on theoretical grounds one would expect
to find them numerous.   In 'tailed cells' the centrosome, instead
of fragmenting or forming a multipolar spindle, sends out a long,
somewhat spiral filament that grows outside the cell like the axial
fiber of a spermatid.   These filaments are extremely delicate struc-
tures and difficult to make out.   The stage at which these axial
fibers grow out from the centrosome may vary somewhat.   In figure
23 the filament had evidently formed during late diakinesis, as
there is a nuclear wall present.   Figure 22 shows the fiber extend-
ing out from the periphery of the nucleus—an unusual condition.
Some of these figures correspond to, in fact are practically identi-
cal with, Broman's ('00) drawings of giant spermatids in adult
Bombinator ingenus material.   Compare my figures 22 and 23
with his figures.

The type of cell represented in these figures is very abundant
in larval material, especially following the period of greatest abun-
dance of aberrant spermatocyte divisions.   Not all such cells show
axial filaments, indeed, they are rare.   Such cells with filaments
growing from the centrosome may be regarded as giant spermatids
resulting directly from transformed first spermatocytes which have
not undergone either first or second maturation division.   Compar-
ison of the stages figured in plate 13 brings out this point clearly.
The same type of cell but without axial filaments is quite abun-
dant; these originate in the same manner as described above, but
cannot be spoken of as spermatids in the absence of the axial
fibers.   Broman has observed several filaments growing out from
a single cell in his adult toad material; so far such cases have not
appeared in my material, and it has been a source of some wonder
on my part why such cells are not of greater frequency.   Cellu-
lar conditions in the first-season larvae are ideal for the devel-
opment of such structures in abundance.   The relative infre-
quency of the tailed cell may perhaps be correlated with the
fragmentation of the centrosome.

The giant spermatids are non-functional and usually undergo
no further metamorphosis, but degenerate and are resorbed.
Stages in the process are shown in figures 103 to 110.   In very

rare cases these abnormal spermatid-like bodies apparently give rise by condensation of the nuclear material and elongation of the cytoplasm to structures bearing a faint resemblance to the apyrene spermatozoa of certain prosobranchs.

The degeneration of the first-season spermatocytes at the metaphase is somewhat analogous to the degeneration of that type of ova that requires the stimulation of a spermatozoon to enable it to complete the developmental cycle. Mead ('98) and Conklin ('05) both observed ova with perfectly formed spindle and chromosomes go to pieces at this stage without further development unless fertilized. In the case of the larval bullfrog spermatocytes, fragmentation of the centrosome is the immediate cause of the failure of the cells to divide and of the resulting degeneration. Professor Conklin has suggested that perhaps the non-fertilized ova observed by him also go to pieces because of centrosomal fragmentation. It is known that the entering spermatozoan brings in a centrosome which takes part in the cleavage process. In regard to the abortive maturation cycle of the first-year bullfrog larvae it is possible that it is a vestige of an early reproductive cycle inherited from remote ancestors. Centrosomal fragmentation is merely the more obvious morphological cause of the degeneration of the spermatocytes and itself a symptom of a deeper-seated derangement of cellular life.

Cytoplasmic and nuclear changes in the degenerating spermatocytes of first-year larvae

The nuclear changes are more or les characteristic of degenerating cells in general, including those just described as ultimately destined to form the spermatid-like bodies. The initial stage in degeneration apparently first affects the centrosome, the chromosomes and cytoplasm are later attacked. To take a typical example of degeneration in a spermatocyte (omitting those polyasters where the chromosomes are pulled to pieces), there is first fragmentation of the centrosome and formation of polyasters, followed by shortening and thickening of the tetrads accompanied by increased staining capacity. The chromosomes soon lose the ring-tetrad structure, and the annular space entirely dis-

appears as though there had occurred a running together of the chromosomes. The lugs or knobs marking the synaptic ends of the chromosomes round off and disappear, leaving an oval-shaped shiny mass, resembling a heavily stained oil drop. Such masses tend to run together, forming larger units, until in final stages of the process the original fourteen tetrads are represented by three or four large deeply staining spherical masses (fig. 22 and 23). During this time the nuclear wall may or may not have disappeared, depending upon the age of the spermatocyte when degeneration began. Where this process sets in after spindle formation there is no nuclear wall. In cases where the nuclear membrane is present, the entire nucleus is excentric in position (fig. 106). In those cases where degeneration begins after the complete formation of the mitotic figures, and after the tetrads are arranged on it, the history of the degeneration is slightly different from that just described. The essential difference is that there is no nuclear wall present, the spindle apparatus is resorbed into the surrounding cytoplasm, and the tetrads go to pieces in situ. The latter take on the appearance of oily masses which may or may not fuse together. In final stages, all that remains of the fourteen tetrads and mitotic figure is a group of oily vacuoles grouped together much in the same fashion as the chromosomes were grouped on the spindle. In many instances the number, and even the size relations of the vacuoles corresponds to the first-maturation chromosomes (figs. 109 and 110).

The cytoplasmic changes accompanying these regressive nuclear phenomena are interesting. The normal, clear, lightly staining protoplasm becomes yellow in color, much vacuolated, and numerous spherical droplets of yolk-like substance appear. In this connection it is interesting to note that an essentially similar yolk-like material has been described and figured by practically all workers on apyrene spermatozoa. For instance, Gould's ('18) description and figures for Crepidula plana and Reinke's ('12) figures of Strombus. However, as the substance in question occurs in degenerate and functionless cells in all cases, the presence of similar degeneration by-products is to be expected and has no special significance. The writer doubts if this substance is yolk, though it does resemble it.

Maturation cycle of second-year larvae and formation of functional spermatozoa

Much that should more properly have been discussed in this section has been referred to here and there earlier in this paper in order to clear up certain sources of confusion which might arise.

The second-year larval sexual cycle differs from that of the first year in two ways: 1) The germ cells of the second maturation cycle are considerably smaller, the tetrads are consequently much smaller than those of the first maturation cycle, and have less the appearance of rings than of dumbbell-shaped bodies when attached to the first maturation spindle; 2) mature spermatozoa are produced, there is little cell degeneration, but few polyasters occur, and hence few cases of fragmentation of the centrosome. All maturation divisions are normal. It is obvious that there is a vast difference between the first and second maturation cycles of the male larvae; the first is aberrant, the second normal; one culminates in degeneration, the other in the production of functional male sex cells.

The smaller size of the germ cells of second-year larvae is not difficult to explain. During the period of the first sexual ripening practically all of the germ cells in the gonads are affected, and consequently destined to degenerate and disappear. There are, however, a few primary spermatogonia with polymorphic nuclei, lineal descendants of the primordial germ cells, scattered here and there through the gonad, which fail to undergo the precocious maturation cycle. These cells are generally, though not always, found near the sex cord region. In the interval between the first and second larval sexual cycles these cells apparently divide rapidly and spread through the gonads. It is probably the repeated division of these cells, and their progeny that brings about the marked reduction in cell size, so noticeable at the second maturation cycle. The proliferation of germ cells is so extraordinarily rapid in the gonads of tadpoles just about to metamorphose that the cellular size becomes reduced to a size scarcely larger than that characteristic of the larger stroma or peritoneal cells. Indeed, conditions are such in

the gonads at this time, and especially in certain individuals, that a great many of the definitive sex cells appear to arise by an actual transformation of mesothelial elements into germ cells. This question is still under investigation, for it is exceedingly difficult to determine definitely whether this is or is not the case from morphological data alone. Certainly in my material there is very suggestive morphological evidence that such transformations may possibly occur, but whether such transformations actually do occur is an entirely different thing.

Another factor to be considered in regard to the reduction in cell size of the first-year germ cells is the fact that the entire gonad undergoes a striking diminution in size during the second year of growth, taking on the character of the adult testis. There is a great loss of water from the tissues at the time of metamorphosis and consequent shrinkage of the cells of the animal in volume.

The size of the chromosomes depends upon the volume of the surrounding cytoplasm and of the nucleus, hence the smaller size of the second maturation cycle tetrads. These tetrads are of the short dumpy type found normally in adult frogs and toads (figs. 120 to 128).

The interesting fact that the sex products of the first larval sexual ripening are all abortive, while those of the second larval cycle are normal is something of a puzzle, and the only explanation occurring to the writer is based upon the phylogenetic history of Anura and will be discussed later in this paper along with some data of a somewhat similar nature regarding mammals and birds.

There is one type of cell in the gonads of second-year larvae and metamorphosed frogs that may remain about equal in size to the germ cells of the younger larvae—the primary spermatogonia. This type of cell is large in frogs and larvae of any age.

During the month of August, 1919, several very large tadpoles were captured with a total length of 140 to 160 mm. Examination of the testes of male individuals showed many normal spermatocyte divisions, spermatids in all stages of development, and a few mature spermatozoa. At this time the efferent ducts of the testis were not yet fully developed. The gonads were extremely small and immature looking. Female gonads of larvae of similar size showed only oöcytes undergoing growth.

The age of these animals could only be estimated by their size and developmental stage, and were probably about two years old. Some of the tadpoles would have undergone metamorphosis within a short time but for the lateness of the season. The writer has known for several years that young male bullfrogs shortly after metamorphosis are sexually mature, though in regard to size they are pygmies compared to the adults and it is difficult to see how they could possibly copulate with mature females. The female Rana catesbeiana apparently does not become sexually mature and ready for copulation until several years after metamorphosis, and in this respect resembles the European frogs, such as Rana esculenta. According to the observations of R. Hertwig, Witschi, and others, the female of Rana temporaria and Rana esculenta does not become fully mature and ready for copulation until the fifth season after metamorphosis. Despite the fact that the females are sexually immature, the young male bullfrog apparently ripens his sexual products continuously, beginning with the first year of larval life, though the first-year sexual cycle is abortive.

The writer has observed somewhat similar phenomena in the leopard frog, Rana pipiens. It is not uncommon to find very small immature looking individuals of this species with sperm in their testes. It has been known for several years that prolongation of the larval life of tadpoles of this species by thyroid extirpation does not prevent the normal seasonal ripening of their sex products. Such ripening corresponds to the second larval sexual cycle in Rana catesbeiana, for it is probable that the tadpoles of this species (i.e., Rana pipiens) undergo a very precocious and abbreviated maturation cycle very early in larval life. This early cycle in Rana pipiens would correspond to the first sexual cycle of Rana catesbeiana; the prolonging of the larval life of the leopard-frog tadpoles leads to the second seasonal ripening and production of normal spermatozoa just as in the bullfrog, though in the latter species the period of larval existence covers the second seasonal ripening of the germ cells.

The germ-cell cycle of the second year in Rana catesbeiana larvae, as was previously stated, is normal in every way, hence no description will be given of the process here. The secondary

spermatocytes give rise to normal spermatids and some sperma-
tozoa, and the writer has nothing to add to this phase of the sub-
ject that has not been described many times before in papers
concerned with the spermatogenesis of other amphibians.
Unquestionaly, these larval sperm cells are functional, because
morphologically they are indistinguishable from spermatozoa of
adult frogs (figs. 118 to 131).

DISCUSSION OF OBSERVATIONS

1. Amitosis in anurans

It has frequently been stated by earlier investigators working
with amphibian material that amitosis occurs quite commonly in
the testis cells of urodeles and anurans. Several writers have even
asserted that at certain seasons of the year amitosis is the sole
method of division (La Valete St. George, Meves, Benda and
McGregor). It has even been seriously stated that the primary
spermatogonia not only divide amitotically, but the results of
such direct division become functional spermatozoa. Meves and
Benda state that amitosis occurs by means of the constrictive
force of a ring-shaped centrosome in Salamandra. McGregor
states that the nucleus is divided by a cleft into two approxi-
mately equal parts. Oddly enough, in view of these positive
statements, the writer has never observed anything in primary
or secondary spermatogonia or in follicle or stroma cells that is
comparable in any way to amitosis. The polymorphic nuclei of
the spermatogonia do somewhat superficially seem to be con-
structed into two halves at times, and the constriction may be
deep enough to give (fig. 1) the appearance of separate nuclei
in the same cell. Careful study reveals connecting portions lying
at deeper levels. It is evident from descriptions of amitosis in
amphibians that polymorphism of the nucleus has been mistaken
for direct division. The writer takes the position, perhaps ex-
treme, that in the spermatogenesis of anura, amitosis does not
normally occur, and if it ever occurs in these forms, it is an
extremely rare and aberrant condition, save in senescent cells
such as those of Bidder's organ, and even in this degenerate struc-
ture direct division is uncommon.

2. The polymorphic nuclei of amphibians

Practically all investigators of the germ cells of urodeles and anurans have described and figured the bizarre and striking lobulation of the spermatogonial nuclei of these forms, but few have attempted any explanation of the peculiarity. Most of the earlier investigators regarded the nuclear polymorphism as stages in amitotic division. The writer has observed, however, that the striking nuclear lobulations of the spermatogonial nuclei in Rana catesbeiana larvae, are nothing more or less than chromosomal vesicles and fusions of such vesicles. In reality, the polymorphism of nuclear structure is due to these vesicles, remaining more or less distinct and independent from one another. That this view is essentially correct is readily seen by examination of early prophase and late telophase spermatogonial divisions. In early prophases the individual chromosomes arise by condensation of the chromatin material of the vesicles. Later telophases clearly show the formation of the vesicles which increase greatly in size as the cell grows.

Conklin ('02) has called attention to the occurrence of such chromosomal vesicles in Crepidula. He states in this regard:

1. The chromosomes, consisting of chromatin enclosed in a linin sheath, divide and move to the poles of the spindle, where they partially surround the spheres. 2. Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus-like body, while the wall remains chromatic. 3. These vesicles continue to enlarge and then unite into the 'resting' nucleus. The nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as achromatic partitions.

Again on page 47 of his ('02) communication, Conklin writes:

It sometimes happens, especially in eggs in which more than the normal number of centrosomes and asters are present, that some or all of the chromosomal vesicles do not fuse, but remain distinct through the whole of the resting period. In such cases each of the vesicles behaves like a miniature nucleus, absorbing achromatic material and forming a net-work of chromatin either within the vesicle or on its walls. In this growth and differentiation the vesicles keep pace, step by step with the normal nucleus, so that one must regard the resting nucleus as virtually composed of vesicles, though their union may be so intimate as to hide this structure.

The resting nucleus is not, therefore, a single structure any more than is the equatorial plate. It is composed of units, each of which, so far as known, has the properties of the entire nucleus, and the union of these vesicles into a single one may be considered as a secondary character. It is altogether probable that the chromosomes, and hence the chromosomal vesicles preserve their identity throughout the resting period, and I venture the suggestion that the daughter chromosomes will be found to arise within the chromosomal vesicles.

The description just quoted, of the formation and behavior of chromosomal vesicles in gasteropod molluscs, applies equally as well to the conditions in the bullfrog larva, and certainly cannot be better stated than Professor Conklin's description (figs. 1, 2, 3).

Häcker ('95) reported that the chromosomes of the early cleavages of Cyclops brevicornis formed two groups of vesicles, one group from the paternal, the other from the maternal pronuclei.

More recently, Wenrich ('16) has reported that each chromasome in Phrynotettix becomes surrounded, as early as the anaphase, by a hyaline region; that this region expands in the telophase; that the chromatin of each chromosome becomes diffused within its own region; that a membrane becomes formed at the boundary between the hyaline region and the cytoplasm, producing the chromosomal vesicle. The nuclear membrane consists of the outer walls of the vesicles at the periphery of the nuclear group. Wenrich concludes that the hyaline region is formed at the expense of the cytoplasm and that the material of each chromosome tends to remain within the space of its own vesicle, a core of chromatin being particularly noticeable in the center of this region, and that the prophase chromosome subsequently formed, is developed out of the substance of one, and only one, of the previously existing telophase chromosomes.

Conditions in Rana catesbeiana larvae, while not so clearly marked, in regard to individual chromosome vesicles, as those described for Orthopteran material, nevertheless strongly indicate that the 'polymorphic' nucleus of amphibians is nothing other than a group of large chromosomal vesicles, more or less independent, the outer walls of the outermost vesicles forming the nuclear membrane.

Some of these vesicles are of exceedingly large size in the primary spermatogonia, and represent possibly, two or more individual vesicles pressed so tightly together as to appear as a single vesicle. At times the individuality of the larger number of vesicles is obscured or may disappear altogether, not to appear again until the early prophase of the succeeding division when the chromosomes reform within the vesicles. Figures 1 and 2 give a good idea of the enormous size attained by these vesicles in certain cells. The combined size of the vesicles is so great that the nucleus completely fills the cytoplasm except for a narrow peripheral border.

These chromosomal vesicles are the means by which the individuality of the chromosomes is maintained from cell generation to cell generation. During the so-called 'resting' stages of the cell, when the chromosomes appear to have lost their identity, and merged with the other elements of the nucleoplasm, they are in reality diffused within little sacs or vesicles, and probably thus remain entirely separated from one another throughout this diffuse period. No one nowadays seriously maintains that chromosomes maintain a strict morphological identity, i.e., appearance, throughout all stages of cell life, but that they do maintain a genetic continuity or individuality throughout 'resting' stages of cellular life by means of chromosomal vesicles cannot be seriously questioned.

The chromosomes that arise from chromosomal vesicles during prophase stages of mitosis are the same chromosomes that went into them during the telophase of the preceding division. The writer can speak only for the conditions presented by the frog, but the accounts in the literature indicate that this statement probably holds true for a great many forms, possibly all. The fact that such chromosomal vesicles have not been found in certain groups, as for instance, coelenterates, according to G. T. Hargitt ('20), is no sure indication they do not exist in that group.

3. Synapsis

This question needs little discussion here, considering the beautiful work of Janssens ('05), Janssens and Willems ('08), the Schreiners ('06), and Snook and Long ('14) on various amphibian types. The conditions in Rana catesbeiana larvae are essentially similar to those described by these works for the urodeles.

Some of the earliest observations on the problems of synapsis were made on amphibian material, chiefly urodeles, and an end to end conjugation of chromosomes or telosnapsis was generally conceded to occur. Janssens ('05), working with Batracoseps, demonstrated parasynapsis, and later, working in collaboration with Willems, showed this to be true of the anuran Alytes. Wilson ('12) after a study of Janssen's material, agrees with this author regarding parasynapsis in Batracoseps. Montgomery ('11) who previously ('03) had described telosynapsis in urodeles, reversed his earlier opinion and states (p. 753): "During the past year I have also convinced myself of the occurrence of parasyndesis in Plethodon, such as Janssens had described for this object and the Schreiners for Salamandra." Snook and Long ('14) describe the same kind of evidence for parasynapsis in Aneides lugubris as that presented by Janssens and the Schreiners.

It is interesting to note that King ('07) denies the existence of parasynapsis for Bufo lentiginosus, and states that telosynapsis is the method of chromosome pairing in this form. King regards the period of synapsis in Bufo as occurring coincidently with synizesis—a condition now generally regarded as an artifact, at any rate in Amphibia. The tetrad chromosomes of the first maturation mitosis, King thinks, arise by transverse segmentation of the thick spireme. If this description of conditions in the toad is correct, then this form differs markedly from other amphibians. Recently, however, the writer has had an opportunity of examining preparations of Bufo, and is convinced that parasynapsis occurs in this animal as the normal method of chromosome conjugation. Amphitene stages are abundant in the material examined by me, and this is the true period of synapsis in anurans (figs. 11, 12, 13, 36 to 38).

The twisting together of the leptotene threads to form the double pachytene spireme which occurs during the amphitene in anura (fig. 12) seems to the writer to be the period when the mechanical conditions for the 'chiasma-type' theory of Janssens are present, and not during the later stage figured by this writer. This theory of 'Chiasma-type' has been extensively employed by Professor Morgan and his co-workers to explain 'crossing-over' in Drosophila. It has repeatedly been observed that genetic factors belonging to a certain group, and presumably carried by a single chromosome, go into a mating together, but do not always reappear together, as they should, if carried by a single chromosome that has maintained its individuality throughout. Janssens endeavored to explain the anomalous genetic behavior of such factors on mechanical grounds, i.e., by showing that in the behavior of the chromosomes, at certain stages in the maturation cycle, it is possible for actual 'crossing-over' of parts of homologous chromosomes to occur, and this exchange of parts of chromosomes he termed 'Chiasmatype.' This theory is based upon a study of certain postspireme (strepsinema) stages in the spermatogenesis of the urodele Batracoseps. In this form, after the secondary longitudinal split (equational split) has taken place, the tetrads are composed of four separate strands or chromatids. These strands may cross each other at certain places, and, owing to strains or weakness at the point of contact, break, subsequently recombining in such a manner as to form threads composed of parts of both original strands. That is to say, parts of the two strands 'crossed over' and became incorporated as a portion of the opposite chromatid. Janssens has carefully figured many such apparent 'cross-overs' in the postspireme stages of Batracoseps.

There can be no reasonable doubt of the accuracy of the genetic evidence for 'crossing-over,' nor of the general truth involved in the chiasma-type theory. The point to be considered here is whether or not the cytological evidence for this view is not more convincing if a stage in the maturation cycle of the chromosomes is used as the basis of cross-over, earlier than the early tetrad state employed by Janssens. The mechanical conditions furnished by the amphitene period in the bullfrog, for crossing over of

parts of homologous chromosomes is well-nigh perfect, certainly more so than the later stages. In the first place, the side-by-side pairing of the two leptotene threads is accomplished by a process of twisting together, and not merely by a side-by-side union; secondly, the twisting of the homologous threads is so tight that all trace of their double nature is lost and the two elements appear as one. It is only later at the time of separation during the diplotene that the dual character of the pachytene thread again becomes apparent. It would be odd if during the process of separation of the tightly twisted threads 'crossing-over' did not sometimes occur (figs. 11, 12, 13, 36 to 38).

Aside, however, from the ideal conditions presented by the amphitene stage for exchange of parts of chromosomes, the period figured by Janssens as furnishing actual cytological evidence of such exchange is not entirely satisfactory. The chief objection here is that study of the postspireme chromosomes of the maturation cycle in the larval bullfrog fails to support the contention that breaking and recombination of the chromatids occur at this particular period. The strepsinema stages of the bullfrog tadpole are very similar to those figured by Janssens, but the writer is of the opinion that crossing-over does not occur here; at any rate, no good evidence for it has been observed. The tetrads are of the non-cross-over type like those figured by Wenrich ('16) and Robertson ('14) for grasshoppers.

Strepsinema stages of tetrad formation are perfectly definite and characteristic periods in the maturation cycle, and so far as my material is concerned, the chromatids appear to preserve their identity through this period (figs. 16, 40 to 63).

There is certain genetical evidence indicative of chromosomal 'crossing-over' during the early synaptic stages of the oöcytes of Drosophila, such, for instance, as Plough's ('17) experiments. He found that environmental changes such as low or high temperature markedly increased the percentage of 'cross-overs' in the second chromosome of Drosophila melanogaster (ampelophila). The temperature apparently increased the amount of 'crossing-over' at a definite stage of oogenesis, and Plough's evidence suggested strongly that the chromosomal exchange takes

place at the stage when the chromosomes of Drosophila are known to be finely drawn-out threads. In other words, he localizes the period of 'crossing-over' in the stage of oogenesis when twisting together of the homologous threads is possible.

It matters little, in so far as the validity of the genetical evidence is concerned, at exactly what stage in the germ-cell cycle 'crossing-over' may take place, for that such a process does occur can scarcely be denied in view of the mass of positive evidence. It is not impossible that the phenomenon may take place at several different stages.

It is an odd fact that 'crossing-over' of genetic factors apparently does not occur in the male Drosophila, but is confined solely to the female. Oddly enough, the chiasmatype theory invoked to explain it is based upon conditions observed in male Amphibia. So far no one has advanced a satisfactory explanation to account for the apparent absence of this phenomenon in the male Drosophila. Nabours has reported evidence for 'crossing-over' in the males of grouse locusts, Castle for the male rat, hence it is evident that it is not confined solely to females.

4. The chromoplasts: (?) karyosomes

Regarding the true nature of these bodies and their relation to the chromosomes, the writer is in doubt, Janssens ('05) who has made a careful and detailed study of the origin and fate of these structures in Batracoseps states:

> Que le chromoplaste prend naissance aux derniers télophases spermatogoniales et qu'il résulte d'un empâtement dû an dépôt d'une substance sidérophile entre les pointes des V chromosomes aux pôles de la figure.
>
> Qu' à mesure que le chromosome se remplit de substance sidérophile le chromoplaste diminue de volume. Il est donc naturel de la considérer comme une substance destinée à être absorbée par les chromosomes à la fin du stade auxocytaire comme il semble qu'elle à être excrétée par eux au commencement de ce stade.

This view is an interesting one; however, the writer has not paid sufficient attention to the chromoplasts and nucleoli in Rana catesbeiana to make any statement regarding the origin and fate of these structures.

The view of Eisen ('00) that the definitive chromosomes of the spermatocyte are derived from the chromoplasts and that "chromoplasts guide the formation of the chromosome just as the archosomes guide the formation of the spindles," does not seem to be entirely substantiated by conditions in the bullfrog tadpoles. In this form the chromoplasts appear to have little to do with the origin of the definite chromosomes in so far as the chromatin material is concerned, for this originates from the preexisting chromatin blocks of the last spermatogonial telophases by a spinning-out process of the leptotene threads. But it is very likely, and my own observations bear this out, that the chromoplasts do give up substance to the chromosomes, though just what the nature of this substance is the writer is unable to say. It is doubtful if the chromoplasts are composed of true basi-chromatin.

In early stages of chromosome formation, such as the preleptotene and leptotene, the chromoplasts are usually large heavily staining bodies, to which are attached several chromatin threads. As development of the threads proceed, the chromoplasts become smaller and take the stain with less avidity. In still later stages they become vacuolated as if being drained of their contents by the growing threads. In final stages these bodies disappear.

5. Significance of the maturation cycle in the larvae

It is possible that the precocious, seasonal ripening of the male germ cells of larval bullfrog represents a recapitulation in ontogeny of a primitive, phylogenetic sexual cycle of ancestral forms, when the Anura were sexually mature and reproduced as larvae, much in the same fashion as does the axolotl to-day. Few biologists would hesitate nowadays to deny that the latter is not merely a neotenous, gigantic, sexually mature larva of the urodele Amblystoma tigrinum, in view of the work of Chauvin ('75) and Duméril ('65). The question why this animal sometimes fails to undergo metamorphosis in certain districts does not concern us here (Swingle, '19). Besides the axolotl there are numerous other instances on record of neotenous, sexually mature amphibians that have failed to metamorphose at the proper time. So far as the writer is aware, such individuals are confined

to the urodeles, with the exception of the bullfrog larvae described here. In other anurans permanent retention of larval characters may be experimentally produced by prolonging the larval life by thyroid extirpation; the retention of the larval somatic characters has no effect upon the germ cells. Similar results were obtained by the writer (Swingle, '17–'18), where it was shown that acceleration of metamorphosis by thyroid feeding does not accelerate the normal course of events in the germ-cell cycle.

It appears possible that the precocious seasonal ripening of the male tadpoles germ cells is a recapitulation, just as the tadpole soma is possibly a recapitulation of an earlier phylogenetic stage when the present-day Anura were more like the Urodela than they are at present, both in regard to body form and sexual conditions. It would be interesting to know whether or not larval urodeles show any such precocious sexuality as described here for anurans. It is not improbable that other vertebrates with larval periods of development, such as some of the eels and petromyzonts, will be found to present analogous conditions to those described for the bullfrog larva. In fact, judging by certain facts to be presented hereafter, it seems likely that all the vertebrates present some such precocity of the germ-cell cycle as described here. If the phenomena described here are in any way rooted in past phylogenetic conditions, it is a much more remote past than anything represented by any living Urodele type.

6. Is there a precocious sexual cycle in other anurans?

This question must be answered at once in the affirmative.

In Rana catesbeiana the larval period is the longest of any other anuran known, and, as a consequence, the precocious sexual cycle of the tadpole is carried farther than in other frogs. There is no question but that if other anurans presented conditions in their germ-cell cycle as marked and unmistakable as those in the bullfrog, such conditions would have been reported years ago. One could not easily overlook cysts of spermatocytes in which the tetrads are of sufficient size to permit counting with a one-sixth Leitz objective and a no. 5 ocular. Although conditions in other frog species are not so plain and easy of interpretation as those presented by the bullfrog larvae, yet nevertheless such species

apparently show essentially identical phenomena as described by myself. The trouble heretofore has been one of interpretation. In Rana temporaria and Rana esculenta tadpoles the same precocity of the sexual cycle, as is presented by the bullfrog has been described many times by various investigators of these European frogs, but has been interpreted in a manner entirely different from the explanation here given. The figures and descriptions of Bouin, Kuschakewitsch, Witschi, Schmidt-Marcel, and others on the history and development of the germ cells of these frogs plainly indicate the precocious maturation process in the larvae. However, the ripening of the tadpole germ cells of the species studied goes only up to and including the pachytene, according to their figures. These writers probably misinterpreted the sexual conditions, and this has led to some bizarre theories of sex differentiation in frog larvae. According to the interpretation of this school, all larvae whose germ cells presented auxospireme, i.e., leptotene and pachytene maturation stages, are to be regarded as females, because it is only female animals that show such maturation changes in larval or embryonic life. Similar maturation stages of male sex cells do not occur until near the period of sexual maturity, according to them. In the anurans studied by these writers the precocity of the sexual cycle is very marked, and the germ cells do not go beyond the pachytene and form tetrads and first-maturation spindles as normally occurs in Rana catesbeiana. Consequently they were not aware that the male larvae exhibits a precocious maturation cycle coincident with that of the females, when the cells of the latter go through the early stages of oöcyte formation. As a consequence of this developmental peculiarity, i.e., curtailment of the maturation cycle to the early stages of the process, without exception these writers, being unable to differentiate male from female, concluded that all frog tadpoles first develop as females, then later half of the female tadpoles must transform into males, because the sex ratio of adult frogs is approximately 50–50.

Their conclusions were logical enough, even though probably erroneous, considering their premise that early maturation stages, leptotene and pachytene, are solely characteristic of female animals, when found in immature or embryonic forms. From a

study of the serm cells of Rana pipiens the writer arrived at essentially the same conclusions, and only after a study of the germ-cell cycle of the bullfrog was it possible to unravel the puzzle. The writer does not believe that females transform into males or vice versa, nor that tadpoles develop solely as females during early stages. A correct interpretation is possible only by comparing the bullfrog tadpole with other forms. In the germ-cell cycle of the larvae of most species of anurans, for example, forms like Rana pipiens with short periods of larval life, the precocity of the maturation cycle is apparently very marked and evanescent, hence it is more obscure and difficult to interpret than that of the bullfrog. But perhaps the most remarkable example of precocity of the larval germ-cell cycle is presented by Bufo. In the toad the early maturation phenomena of the germ cells, i.e., leptotene, amphitene, and pachytene stages, appear in extremely young larvae, about two weeks after hatching and are confined chiefly to the anterior end of the male and female gonads, i.e., that portion which develops into the organ of Bidder. The ripening process does not go beyond the pachytene stage apparently before the cells become senescent. The question whether or not Bidder's organ is or is not a rudimentary hermaphrodite gland is reserved for discussion in another paper. A very brief and curtailed sketch of the writer's view of this structure in the toad and the so-called hermaphroditism of the Anura will be found in the American Naturalist for July-August, 1920.

There appears to be some sort of correlation in anurans between length of larval life and stage in development to which the ripening of the germ cells is carried before degeneration. The whole problem of sex development and sex differentiation of anuran larvae needs reinvestigation in the light of these observations on the bullfrog tadpole.

7. Significance of degeneration of sexual elements derived from primordial germ cells

It is with considerable reluctance that the writer touches upon this particular phase of the problem in this paper, which is to be regarded as a cytological introduction to a more extensive report

later upon the entire developmental history of the male and female sex glands and cells in anurans. The problem stated in the heading of this section is of such importance as to deserve a much more detailed discussion than is possible here. However, a brief statement of the more important theoretical considerations suggested by the results obtained in the study of the larval sexual cycle of the bullfrog cannot well be avoided. There are certain obscure and little-known phenomena occurring in several other classes of vertebrates of a similar, if not identical nature with those reported here for the Anura. When the germ-cell cycle of some of the higher vertebrates is correlated with the maturation cycle of the larval frog, there is much that suggests to the writer that possibly we are here dealing with a fundamental principle in germ-cell development, of widespread, perhaps of universal occurrence among the vertebrates. Let us examine some of this evidence.

There are two important theories concerning the origin of the germ cells of vertebrates, each backed by considerable amounts of evidence not lightly to be disregarded: 1) The first view is that the definitive sex cells of the gonads are derived from primordial germ cells which have originated elsewhere in the organism, probably from entoderm, and have migrated into the genital ridges and there differentiated into oöcytes or spermatocytes as the case may be. These primordial germ cells of the embryo are distinct from other surrounding mesothelial cells and have a separate origin from cells of this type. The advocates of this view are many, and a great deal of valuable data in support of this theory has been collected of late years. 2) The second view regards the germ cells as differentiated products of the germinal epithelium, i.e., that they arise by direct transformation of sexually indifferent cells of mesodermal origin.

The advocates of the first point of view, we may, for the sake of convenience, term the 'entodermists,' though not all believers in the Keimbahn theory are agreed that the germ cells take origin from entoderm; the adherents of the second theory we shall call 'mesodermists.' Between these two groups there is little or no common ground, but instead a great deal of controversy.

The first view can be traced back to Nussbaum ('80) who claimed that germ cells are not derived from the soma, but are early differentiated segmentation products which take no part in body formation and retain their primitive embryonic character. Many facts supporting this view have come to light through study of the origin of the germ cells in almost all classes of vertebrates. There can be no question that in most vertebrates the sex cells are early set aside during development, and later migrate into the germ ridges. In the embryo frog this process is readily traced through every stage; the same is true of other forms, also, as, for instance, certain ganoids, reptiles, and birds.

The second or mesodermal view, viz., that the germ cells arise from an original sexually indifferent germinal epithelium originated with Waldeyer ('70) and has been held by a great many observers to be the true method of germ-cell origin. Oddly enough, practically every animal claimed by the 'entodermists' as illustrating their view has also been claimed by the 'mesodermists' as illustrating their theory. For instance, take the Amphibia: Allen, King, and Witschi hold the view that the primordial germ cells arise from the entoderm and give rise to the definitive sex cells; on the other hand, Semon, Bouin, Dustin, Kuschakewitsch, Champy, and Gatenby are all equally certain that the definitive sex cells of the amphibia arise from the germinal epithelium. These views are diametrically opposed, consequently both cannot be either entirely true or entirely false, and the problem is to point out if possible the source of confusion. To take the case of the frog again, in this form the primordial sex cells unquestionably arise from the entoderm. There is absolutely no indications of germ cells arising from the germinal epithelium in early larval stages. The two types of cell in the gonad, mesothelial and sexual, are entirely distinct and it would be difficult to confuse them. All increase in germ cell number is by mitotic division of preëxisting, differentiated primordial germ cells. This state of affairs persists until the tadpole is about 45 to 60 mm. total length. Thus far it is obvious that the evidence derived from the frog is decidedly in favor of the 'entodermists.' However, practically all of the germ cells derived from the ento-

derm in the male bullfrog larva undergo a precocious and abortive maturation cycle ending in degeneration and absorption. A very few descendants of the primordial line of cells fail to maturate or degenerate, and apparently give rise to a new generation of sex cells in the tadpole. Whether or not this second generation of germ cells is derived entirely from the few left-over cell descendants of the primordial line is difficult to say, for in my material at this time there is a marked increase of germ cells which looks suspiciously like an active transformation of epithelial elements into sex cells. The new germ-cell generation undergoes another maturation cycle at the time of the metamorphosis of the tadpole, or very shortly afterward, and gives rise to normal sex products. Thus it is to be seen that the advocates of the germinal epithelium theory may not be entirely mistaken, for in the light of events in the germ cycle of the Anura, it is not sufficient to trace the primordial sex cells into the genital ridges and there leave them. Without following their later history, it is unjustifiable to assume that they do give rise to the definitive sex products. This is what most workers on the origin of germ cells in vertebrates have done. On theoretical grounds the writer doubts if mesothelial cells transform into sex cells but the morphological evidence from my material does not rule out the possibility of such transformations.

The evidence presented by the precocious sexual cycle of the bullfrog leads the writer to believe that further investigation may perhaps show the existence of analogous phenomena throughout the various groups of Chordata. It may not be too much to state as a sort of working hypothesis for further investigation of germ-cell development in the vertebrates, that most of the primordial germ cells, i.e., those arising in early stages of embryonic development, undergo a precocious and abortive developmental cycle, culminating in degeneration, or else degenerate before the precocious maturation cycle has had sufficient time to manifest itself. Furthermore, that the abortive developmental phenomena are perhaps evidences of a phylogenetic regression in the germ-cell cycle to remote ancestral conditions.

The question immediately arises if such an hypothesis, no matter how tentatively stated, is in any sense justified by evidence

presented by merely a single group of vertebrates. The answer is, that scattered here and there through the literature is considerable evidence of an extremely suggestive nature, derived from study of the germ cells in widely separated groups of chordates, which, when taken in conjunction with conditions existing in anurans, suggest at once the working hypothesis stated above. Some of this evidence will be briefly reviewed here.

In 1908 Von Winiwarter and Sainmont described two proliferations of cells from the germinal epithelium of the cat ovary; the first formed the medullary, the second the cortical cords. According to their account, the germ cells and follicle cells of the first proliferation and the sex cells of the second all degenerate and disappear when the young kitten is only a few months of age. About three and a half to four months after birth, the germinal epithelium of the ovary shows marked activity, and proliferates a third generation of germ cells, from which the definitive sex cells of the adult are derived. This third generation forms the cortex of the ovary in grown animals. In a short paper published at nearly the same time as their monograph they state that the definitive ova of the sexually mature cat are derived either entirely from this third proliferation of the germinal epithelium, or else partly from it and from undifferentiated cells left over from the second proliferation, i.e., those that failed to degenerate. For example, these authors state (p. 616):

Es tauchen nun jetzt in den Epithelhaufen und Strängen der Corticalis kleine Gruppen von Zellen auf, deren Kerne im staubförmigen oder deutobrochen Stadium sind. Diese Formen waren schon seit langer Zeit nicht mehr vorhanden, und da sie den ersten Stufen des Wachstums des Oocyten entsprechen, ist es augenscheinlich, dass sie mit einer Neubildung von Eiern zusammenhängen . . . . Wir glauben bewiesen zu haben, dass in Säugetierovarium nicht nur sämtliche Markstränge, sondern auch alle Eier und Follikel der primitiven Corticalis dem Untergang anheimfallen. Die definitiven Eier entstammen entweder von undifferenzierten Zellen der zweiten Proliferation (Pflügersche Schläuche) oder von Zellen der dritten Wucherung oder Invaginationsepitheles. Es ist uns nicht möglich, wenigstens morphologisch, die Elemente der einen und anderen zu unterscheiden.

Now, oddly enough, all of the embryonic (primordial) germ cells described by Winiwarter undergo the characteristic nuclear

changes of oöcytes, such as leptotene, pachytene, and diplotene, before undergoing degeneration.

Rubaschkin ('12) confirmed the conclusions of Von Winiwarter and Sainmont that the cells of the first and second proliferations in the cat degenerate. In the ovary of the guinea-pig this same investigator observed a third proliferation of germinal cells from the germinal epithelium which occurs before birth and which he considers the source of the definitive sex cells.

Firket ('14) using female chick material, showed that the primordial germ cells pass through the first stages of maturation previous to oöcyte formation, leptotene, pachytene, etc., enter the growth period and then degenerate. They all disappear in the chick fourteen days after hatching. The oöcytes of the cortical zone (second embryonic proliferation) practically all degenerate, although he states that he cannot be sure that they all do. There is a new formation of germ cells in the cortical region, from cells derived from the germinal epithelium, and from these the definitive oöcytes develop; but it is not improbable, at least, that a small number of the primordial germ cells are differentiated into definitive ova. One of the conclusions Firket ('14) draws from his work is of considerable interest from the standpoint of the results on the frog recorded here:

Il faut, donc, morphologiquement parlant, considérer les gonocytes primaires des Vertébrés comme étant un rappel phyolgénique des gonocytes définitifs des classes inférieurs, notament des Cyclostomes et des Acraniens. L'epuisement gradual, dans la série phylogénique des éléments de cette lignée a nécessité l'apparition, au cours de l'ontogénèse, d'une seconde lignée de gonocytes, moins précoces (pp. 330, 331).

Recently there came to my attention an abstract of a paper as yet unpublished by this same author. I shall quote the abstract entire because of the striking similarity of the conclusions of this author, to some recorded by myself in this paper, both independently conceived:

In the testis and the ovary of the chick there are two generations of germ cells: primary germ cells, which appear in very early stages, before the genital ridge is formed, and secondary germ cells, which are derived from the so-called 'germinal epithelium.' The former are able to become oöcytes, or spermatocytes, but while most of them

degenerate, it is not possible to determine if any of them give rise to definitive germ cells, because at a certain stage it is impossible to distinguish the former and the latter from each other. In the white rat (male) the same two generations occur, but primary germ cells degenerate before they reach the period of growth and only secondary cells become the definitive germ cells. That primary germ cells disappear, in the ontogenesis, earlier in mammals than in birds, seems to show that they must be considered as being cells in 'phylogenetic regression.'

Two interesting papers by Kingery on the female white mouse show that the phenomenon of primordial germ-cell degeneration is found in the mouse, and perhaps even more important in this connection is the fact that certain degenerating cells of this primitive germinal line may undergo abortive maturation stages even to the formation of first polar bodies.

This author ('14) in a study of the so-called parthenogenesis in the mouse found that the degenerating primordial germ cells, i.e., those of embryonic origin, undergo a degenerative fragmentation and may even form a first polar body and second polar spindle, and may even break up into fragments with or without nuclei in much the same fashion as described by me for the larval spermatocytes of the frog. It is interesting to compare the figures in Kingery's paper with those of my own in degenerating spermatocytes.

In a later paper by this same author ('17–'18), evidence of the kind described for the cat by Winiwarter and Sainmont ('08) and Rubaschkin ('12), for birds and the white rat by Firket ('14, also '20), and the male mouse by Kirkham ('16) is presented. The first or embryonic set of germ cells in the female mouse pass through early maturation stages, leptotene, pachytene, and diplotene, enter the growth period of the oöcyte, then degenerate. The second generation of germ cells arise from the germinal epithelium after birth and give rise to the definitive sex cells of the adult female. He says:

The evidence shows that all these germ cells formed before birth degenerate and are resorbed, none of them developing into definitive ova. This degeneration takes the form of atrophy and resorption in some cases, but in others there may occur atresia folliculi; accompanied by the formation of a first polar body, and a degenerative fragmentation of the egg-cells, simulating more or less closely a parthenogenetic cleavage.

Kirkham ('16) observed that the primordial germ cells of the mouse first appear on the eleventh day after fertilization. In male embryos these primitive germ cells all degenerate, and none persist by the eighth day after birth. The definitive sex cells of the male arise from undifferentiated epithelial elements according to this account, whereas the definitive oögonia are direct descendants of the primordial germ cells. It will be seen that Kingery's account of the definitive germ cells of the female mouse agrees with Kirkham's account for the corresponding conditions in the male.

Felix states that in the human embryo the primordial germ cells degenerate (no details given) and a new generation of sex cells arises from the germinal epithelium which give origin to the definitive sex cells (Keibel and Mall, Embryology).

Now it is obvious that evidence of this sort obtained by different investigators, working on vertebrate forms as widely separated as amphibia and mammals, must be of some significance. In all vertebrates a definite Keimbahn probably exists; this is certainly true of the frog, but the important question is, do the primitive products of the keimbahn and their lineal descendants in these vertebrate forms early undergo an abortive maturation or developmental cycle which ends in degeneration such as occurs in the bullfrog tadpole? Certainly, the evidence looks suggestive. Apparently in the male bullfrog larvae this precocious sexual cycle is carried further than in any other form so far reported. The figures of Kingery for the female mouse and of Winiwarter and Saimmont for the female cat indicate plainly that the primordial germ cells are undergoing a precocious maturation cycle. These figures show every phase in the maturation cycle of normal eggs, such as leptotene, pachytene, diplotene, and growth of the oöcyte, yet, just as happens in the male tadpole, these early maturating cells degenerate. The same condition is reported in birds. It is difficult to avoid the suspicion that we are here concerned with a fundamental principle of germ-cell development. The question arises, why should practically all of the primordial germ cells of vertebrate undergo an abortive sexual cycle long before the animal is mature and ready for

reproduction, and then degenerate? The evidence from the maturation cycle of the tadpole is again suggestive on this point.

In the tadpole we may assume, in so far as it is safe to assume anything in biology, that the abortive and precocious sexual cycle is possibly a case of phylogenetic regression to ancestral conditions when the Anura were permanently of the caudate type and lived and reproduced normally as Urodele-like creatures. The carrying over into the ontogeny of the anuran larva's sexual cycle of this phylogenetic vestige is not surprising, considering the heavy impress of phylogeny upon the tadpole soma. Though this explanation may be involved with much plausibility to explain the larval sexual conditions of the bullfrog, is it in any sense adequate to account for the apparently analogous germ cycle of the Sauropsida and mammals, forms which do not have a larval period? I believe the same explanation applies to these forms also, and that the precocious developmental cycle and degeneration of the primordial germ cells described in the Amniota differ not in kind, but merely in the degree to which the maturation cycle is carried from the larval sexual cycle of the Anura.

In the Amniota we cannot speak of a precocious and abortive larval germ-cell cycle, but we can speak of an abortive embryonic sexual cycle, which, like that of the tadpole, possibly bears the impress of past phylogenetic conditions. And why not? If the embryo of the higher vertebrates can develop gill clefts and a thousand and one other evanescent phylogenetic vestiges in the course of somatic development, it should not be regarded as extraordinary if the germ-cell cycle likewise presented similar 'ancestral reminiscences,' and did a little recapitulating on its own account.

However, it must be confessed that 'phylogeny,' 'recapitulation,' 'ancestral reminiscences,' and other vague and more or less mystical terms of a kindred nature are after all merely convenient pegs upon which to hang our ignorance. There are immediate physicochemical reasons for the degeneration of the primordial germ cells or their abortive sexual cycle, but what these reasons are is unknown, and in view of a better or more plausible, hypothesis to account for this phenomenon, the one presented above is advanced tentatively.

Another point is worthy of consideration here, and that is the possibility of bringing about some measure of reconciliation between the 'entodermists,' or advocates of the Keimbahn, and the 'mesodermists.' In view of the evidence presented by study of germ-cell origin in all classes of vertebrates, there can be no reasonable doubt that the primordial sex cells are products of the entoderm, and probably migrate into the germ ridges at an early period of development. However, according to the hypothesis advanced here these primordial cells, after a period of multiplication, undergo an abortive developmental cycle and for the greater part degenerate—perhaps, in mammals, entirely degenerate. The new cell generation destined to give origin to the definitive sex cells may possibly arise in part from the germinal epithelium by direct transformation of mesothelial elements. The evidence for this point of view is suggestive, at any rate, judging by reports on conditions in the birds and mammals, and there is little evidence to the contrary, but many pure assumptions.

In the bullfrog the writer prefers to believe that some cells of the primordial germ-cell line persist unchanged through the phase of maturation and degeneration, and ultimately, by repeated mitosis are the chief, and probably only contributors to the cells of the definitive sexual line. There is considerable evidence for this view, because a few primordial spermatogonia or at any rate lineal descendants of these cells can be traced through the sexual cycle easily enough, but it is by no means certain that they are the sole contributors to the definitive line of germ cells.

Thus it appears possible that there is some basis here for reconciliation between the entodermists and mesodermists regarding germ-cell origin and development. The former have been at fault by contenting themselves with tracing the primordial sex cells into the genital glands and there leaving them, with the assumption that they persist and form the sexual elements of the adult organism. The mesodermists, working chiefly on mammals, have for the most part ignored the contributions of the 'entodermists' because they have been unable to trace the germ cells back to the very earliest stages such as described for the lower vertebrates.

No investigation of the germ-cell cycle in the Chordata should be regarded as complete or as being more than a half-truth which does not take into consideration the entire history of the germ-cell cycle, from the origin of the primordial germ cells to the formation of the definitive sexual elements of the adult. The investigators of the keimbahn have not gone far enough, for between the origin of the primordial germ cells and the formation of the ripe sexual products there is a critical stage in the germ-cell cycle, characterized by a precocious and abortive maturation, degeneration, and reformation of a new line of germ cells, perhaps by transformation of mesothelial elements, but more probably by active mitosis of a few left-over cells of the primordial line.

According to Hegner (Germ-cell cycle of animals, p. 99), germinal epithelium theories of germ-cell origin have little if any evidence in their favor, since no one has actually observed a transformation of peritoneal or mesoblast cells into germ cells. "On the other hand there is an abundance of proof that these cells (germ cells) migrate from some distance into the position of the sex glands."

The writer is quite in agreement with Hegner regarding the existence of a keimbahn in vertebrates, but is not so sure that no one has actually observed a transformation of mesoblast cells into germ cells in late stages of development or that germinal-epithelium theories have little if any evidence in their favor. My observations on the larval bullfrog have taught me caution in regard to dogmatizing on this problem. Odd as it may seem, it is not impossible, in the light of conditions described above for the bullfrog larvae, that the primordial germ cells of vertebrates, i.e., the keimbahn elements discussed by Hegner, may possibly be found upon further investigation to contribute little if any to the definitive sex products of the adult organism. Further investigation of the germ-cell cycle of the chordates may possibly enable the 'mesodermists' to turn the tables on the 'entodermists' with a vengeance by showing that no one has actually observed a transformation of keimbahn cells into definitive sex products. Though regarding himself as an entodermist, and taking the point of view that the keimbahn is probably continuous in

vertebrates, and that there is no actual transformation of meso-
thelial elements into sex cells, the writer admits that conditions are
such in the bullfrog that it is impossible to state positively that the
primordial germ cells of the bullfrog tadpole do give rise to the
definitive sex cells of the adult frog. Certainly, this is the more
probable view, though the burden of proof rests with those of us
who hold that the keimbahn is continuous.

It would seem from this that the crux of the whole problem is
to determine whether or not germ cells can develop in an organ-
ism after the primordial germ cells have been destroyed. If
they do develop, then the doubtful question of transformation
of mesothelial cells into germ cells is settled in favor of the meso-
dermists, but if they do not develop, and the gonad is sterile and
remains so up to the period of sexual maturity, then the decision
is in favor of the entodermists. It is not sufficient to extirpate
the primordial germ cells or otherwise destroy them, as was done
by Reagan in the embryo chick, and then report the resulting
sterile gonad as conclusive evidence against the idea of a trans-
formation of epithelial elements, because proof positive can only
be had by rearing the animals to sexual maturity.

The only adequate method of attack upon this problem is by
experimental methods. Morphological methods are not sufficient
to determine whether or not a germ cell in the germinal epithelium
or sex cord tissue is a transformed epithelial element or a small
germ-cell descendant of the primordial line. Transition stages,
nuclear configuration of the cell, size, position, and such like may
be illusory. In my material there is apparently every transition
stage between peritoneal and true germ cells, use whatever
morphological criterion you please, at certain developmental
stages of the tadpoles, and such transition stages almost fill the
gonads, but always the question arises upon examining these
apparent transition stages—they look exactly like mesothelial
cells transforming into germ cells, but are they? If one must
judge from morphological data alone, the answer is that they
could very readily be taken for mesothelial elements transforming
into germ cells, but, as stated before, the morphological criterion
alone does not furnish sufficient evidence to permit one to make
a definite answer.

SUMMARY OF CONCLUSIONS

A. The origin and fate of the primordial germ cells

1. The primordial germ cells of the embryo bullfrog are first distinguishable from other entodermal elements in embryos of 7 mm. total length. They arise from the entoderm as a median ridge of yolk-laden cells just dorsal to the roof of the archenteron, ventral to the aorta, and separating the two lateral mesodermal plates from each other.

2. In embryos of 8 mm. total length, the germ-cell ridge becomes separated from the underlying entoderm forming the roof of the archenteron, partly by the median growth of the two lateral plates which pinch off the ridge and also by active migration of the germinal elements themselves. In cross-section at this stage, the germ cells are found at the root of the forming mesentery as an unpaired ridge, consisting of two or three large yolk-laden cells.

3. As development progresses, this median ridge of germ cells splits longitudinally and the cells of the two halves migrate laterally on either side to form two independent ridges, invested with peritoneum. This stage is represented in embryos of 9.5 mm. total length.

4. The two germinal ridges project into the coelomic cavity and enlarge considerably by increase in number of their cellular elements. The primitive sex cells actively divide and there is also a migration of mesenchymal cells into the ridges from the mesonephros and peritoneum. These conditions are found in 14 to 15 mm. tadpoles. The germ cells have lost their yolk in the meantime.

5. The gonads greatly increase in size. Large cavities are formed, the secondary genital spaces, lined by small non-sexual cells which have migrated into the gonads from the mesonephros by way of the mesentery suspending the gland. When the tadpole has attained a length of 30 mm., the gonads are hollow sacs surrounded by a single layer of peritoneum and one or two layers of germ cells.

6. All increase in the number of germ cells in male larvae up to the 40-mm. stage is beyond question, by mitotic division of the preexisting sexual elements derived from the primordial germ cells of the entoderm ridge. A 40-mm. larva is about one year of age.

7. At the 40-mm. stage, despite the fact the tadpole is an immature larva and the gonads mere hollow sacs and in no way resemble testes, the germ cells enter maturation and pass through every stage of the maturation cycle in a normal manner, up to the first maturation division. In the act of division, the spermatocytes go to pieces and are resorbed.

8. Practically all of the germ cells derived from the primordial sex cells pass through this abortive maturation cycle and degenerate. A very few germ cells lineal descendants of the primordial embryonic sex-cell line persist unchanged, i.e., remain as spermatogonia through the maturation cycle and do not degenerate. Later these few cells give rise to a second generation of smaller germ cells.

9. This second generation of germ cells shortly before metamorphosis of the larvae undergoes a second sexual cycle, characterized by the production of normal spermatozoa. Thus there are two larval sexual cycles: one occurring in immature larva of 40 to 60 mm. and ending in degeneration, the other appearing shortly before metamorphosis, i.e., in larvae 140 mm. total length and ending in the production of normal sex products.

10. In the interval between the first and second larval sexual cycles following the degeneration of large numbers of maturation cells the gonads become filled with small cells which, because of their size, nuclear structure, and staining capacity, appear as transition stages between mesothelial cells (germinal epithelium and sex cord elements) and true germ cells. The later history of these cells shows them to be germ cells, but their origin is open to two interpretations and is not as clear as could be desired. The writer considers these cells as small germ-cell descendants of the primordial sexual elements, and not as transformed germinal epithelium elements, but admits that the evidence from his material is equally strong in support of the germinal epithelium view-point.

11. The sexual cycles of the larval bullfrog are tentatively interpreted, in lieu of a more satisfactory hypothesis, as recapitulations of the germ-cell cycle to past phylogenetic sexual conditions when the vertebrates ripened their sex products at an earlier developmental stage than at present.

12. An analogous precocity of the maturation cycle probably exists in all of the vertebrates, Amniota as well as Anamnia. Evidence for this hypothesis is presented in detail.

B. The chromosomes and larval sexual cycles

1. The diploid number of chromosomes in the male larva is twenty-eight. The elements are J- and V-shaped and curved rods. Portions of certain chromosomes do not take the stain under any circumstances and may give the appearance of fragmenting into two or more parts. Such appearances of fragmentation are illusory.

2. Spindle-fiber attachment is non-terminal.

3. The chromosomes exist in definite pairs according to size and shape, i.e., there are fourteen pairs of homologues.

4. The homologues of any pair are not invariably found side by side within the nucleus, though in general they are near together.

5. The size and shape relations of the chromosomes are perfectly definite throughout all cell generations, and this is probably true not only for the individual, but for the larvae of the species as a whole.

6. As an illustration of the statement just made (number 5), see chromosome pair marked A in figure 6. These chromosomes are peculiar in that the knob-like end-piece is separated from the main body of the chromosome by a clear, non-stainable area. This peculiarity is probably constant in the cells of the larvae, and has been observed in the spermatogonia of twenty-nine individuals of various ages and stages of development.

7. The resting nucleus of the Anuran germ cell is a polymorphic, much-lobulated structure, made up entirely of chromosome vesicles which are incompletely fused, and in many cases the vesicles are

entirely independent. By means of these vesicles the chromosomes preserve their identity through the so-called resting stages.

8. The cells and chromosomes of the larvae are considerably larger than those of the adult frog, and more nearly resemble the cells and chromosomes of urodeles than those of the adult of their own species.

9. This size difference between the larval and adult cells and chromosomes is explained in detail in the text and is considered to be due in part to the number of intervening cell divisions, with reduction of cell and chromosome size the greater the number of divisions, and to reduction in cell size at the time of metamorphosis due to loss of water from the tissues.

10. The haploid number of chromosomes in the larvae is fourteen. The tetrads of the first larval sexual cycle are extremely large and of the open-ring type characteristic of urodeles. They differ markedly from the type of tetrad appearing in the second larval sexual cycle.

11. Conjugation of the chromosomes is by parasynapsis, and occurs in the amphitene stage, when the leptotene threads twist together to form the pachytene.

12. Evidence of 'crossing-over' during diakinesis, such as figured by Janssens for Batracoseps, has not been observed, or rather, has been observed but not interpreted as such. The chiasma-type which appears during diakinesis stages of the bullfrog larvae has been interpreted by the writer as tetrads opening out in two planes at right angles to one another thus giving the *appearance* of 'crossing-over' of the chromatids.

13. It is suggested that 'crossing-over' occurs during the amphitene stage, when the conjugating leptotene threads coil tightly about each other corkscrew fashion.

14. The first larval maturation cycle is normal in every respect save for the size of the cells and chromosomes, up to the formation of the first maturation spindle. The spermatocytes degenerate in the act of division.

15. The cause of the degeneration of the larval spermatocytes is the abnormal behavior of the centrosome which fragments, forming accessory asters and spindles. It is recognized that the

centrosomal behavior is but a symptom of a deep-seated proto-plasmic disorganization of the larval sex cells.

16. Giant spermatid-like structures are formed by the suppression of the first and second maturation divisions and the growth of an axial fiber from the centrosome. These bizarre structures degenerate.

17. The cells of the first larval sexual cycle degenerate and disappear gradually. A few cells, lineal descendants of the primordial germ cells, persist unchanged through the cycle of maturation and degeneration, and give rise by repeated mitosis to a second germ-cell generation in larvae just about ready for metamorphosis. This second cell generation is small in size.

18. Shortly before metamorphosis, this second generation of germ cells undergoes a second sexual cycle, characterized by the formation of normal spermatozoa. The cells and chromosomes are comparable in every way with those of the adult frog and are smaller than the larval cells and chromosomes.

19. The second larval sexual cycle is normal in every respect. There is no degeneration of the sexual elements. The maturation cycle is normal, as are also the spermatozoa, despite the fact the animal is a larva with the efferent ducts of the testis incompletely formed.

20. In so far as the possession of ripe spermatozoa is concerned, the larval bullfrog at metamorphosis may be said to be mature, and in this respect resembles the axolotl.

21. The germ cells of female larvae at the time of metamorphosis are not mature, but are young oöcytes undergoing growth. The writer has some evidence that, like the male, the female larvae may also show a precocious and abortive maturation cycle. This point is now under investigation.

22. The question of hermaphroditism and the sex ratios of the Anura is not dealt with in this paper, but forms the subject-matter of a later communication.

LITERATURE CITED

ADLER, LEO 1916 Untersuchungen über die Entstehung der Amphibienneotenie. Pflüger's Archiv für die gesamte Physiologie, Bd. 164.

AGAR, W. E. 1911 The spermatogenesis of Lepidosiren paradoxa. Quart. Jour. Micr. Sc., vol. 57.

ALLEN, B. M. 1906 The origin of the sex-cells of Chrysemys. Anat. Anz., Bd. 29.
1907 An important period in the history of the sex-cells of Rana pipiens. Anat. Anz., Bd. 31.
1909 The origin of the sex-cells of Amia and Lepidosteus. Anat. Rec., vol. 3.

AMMA, K. 1911 Über die Differenzierung der Keimbahnzellen bei den Copepoden. Arch. f. Zellforsch., Bd. 6.

BALLOWITZ, E. 1905 Die Spermien des Batrachiers Pelodytes punctatus. Anat. Anz., Bd. 27.
1906 Über das regelmässige Vorkommen heteromorpher Spermien im reifen Sperma des Grasfrosches (Rana muta). Zool. Anz., Bd. 30.

BAUMGARTNER, W. G. 1911 Spermatogenesis in the male cricket. Science, N. S., vol. 33.

BENDA, C. 1893 Zellstructuren und Zellteilungen des Salamanderhodens. Verhandl. der Anat. Gesellschaft.

BONNEVIE, K. 1908 Chromosomenstudien. I. Chromosomen von Ascaris, Allium und Amphiuma. Arch. f. Zellforsch., Bd. 1.
1908 Chromosomenstudien. II. Heterotypische Mitose als Reifungs-Character. Nach Untersuchungen an Nereis limbata Ehlers, Thalasema mellita Conn, und Cerebratulus lacteus. Arch. f. Zellforsch., Bd. 2.

BOUIN, M. 1900 Histogénèse de la glande genitale femelle chez Rana temporaria. Arch. de Biol., T. 17.

BROMAN, J. 1900 Über Riesenspermatiden bei Bombinator igneus. Anat. Anz., Bd. 17.
1900 Über Bau und Entwickelung der Spermien von Bombinator igneus. Anat. Anz., Bd. 17.

BRANCA, A. 1907 La spermatogénèse chez l'axolotle. Arch. Zool. Exp. et Générale. Notes et Revues.

CAROTHERS, E. ELEANOR 1916–17 The segregation and recombination of homologous chromosomes as found in two genera of Acrididae (Orthoptera). Jour. Morph., vol. 28.
1913 The mendelian ratio in relation to certain orthopteran chromosomes. Jour. Morph., vol. 24.

CARNOY, J. B., ET LEBRUN, H. 1900 La vesicule germinative et les globules polaires chez les Batrachiens. 2 partie, les Anoures. La Cellule, T. 17.

CHAUVIN, MARIE V. 1875 Über die Verwandlung des mexikanischen Axolotl in Amblystoma. Zeitschr. f. wissensch. Zool., Bd. 25, Suppl., 1875, und Bd. 27, 1876.
1885 Über die Verwandlungsfähigkeit des mexikanischen Axolotl. Zeitschr. f. Wissensch. Zool., Bd. 41.

CHAMPY, C. 1913 Recherches sur la spermatogénèse des Batrachians et les éléments accessoires du testicle. Arch. de Zool., Exp. et Générale, T. 52.

1908 Sur la dégénérescence des spermatogonies chez la grenouille verte. Compt. Rend. Assoc. Anat. Mar.

CONKLIN, E. G. 1897 The embryology of Crepidula. Jour. Morph., vol. 13.

1896 Cleavage and differentiation. Woods Hole biol. lecture.

1901 The individuality of the germ nuclei during the cleavage of the egg of Crepidula. Biol. Bull., vol. 2.

1901 Centrosomes and spheres in the maturation, fertilization and cleavage of Crepidula. Anat. Anz., Bd. 19.

1902 Karyokinesis and cytokinesis in the maturation, fertilization and cleavage of Crepidula and other Gasteropoda. Jour. Acad. Nat. Sci. Philadelphia, vol. 12.

1915 Why polar bodies do not develop. Proceedings Nat. Acad. Sciences, vol. 1.

1917 Effects of centrifugal force on the structure and development of the eggs of Crepidula. Jour. Exp. Zoöl., vol. 9.

DEHORNE, A. 1911 Recherches sur la division de la cellule. I. Le duplicisme constant du chromosome somatique chez Salamandra maculosa et chez Allium cepa. Arch. f. Zellforsch., Bd. 6.

1910 Le nombre des chromosomes chez les batrachiens et chez les larves parthénogenetiques de grenouille.

DUMÉRIL, A. 1865 Nouvelles observations sur les Axolotls nés a la ménagerie. Comp. Rend., T. 61.

EISEN, G. 1900 The spermatogenesis of Batrachoseps. Jour. Morph., vol. 17.

FICK, R. 1893 Über die Reifung und Befruchtung des Axolotl. Zeit. f. wiss. Zool., Bd. 56.

FIRKET, J. 1914 Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. Arch. de Biol., T. 29.

FLEMMING, W. 1887 Neue Beiträge zur Kenntniss der Zelle. Arch. f. mikr. Anat., Bd. 29.

GRÉGOIRE, V. 1905 Les résultats acquis sur les cinèsis de maturation dans les deux règnes, Mém. I. Les cinèsis de maturation dans les deux régnes. L'unité essentielle du processus meitotique. Mem. II. La Cellule, T. 26.

1907 La formation des gemini hétérotypiques dans les végétaux. La Cellule, T. 24.

HÄCKER, V. 1895 Die Vorstadien der Eireifung. Arch. f. mikr. Anat., Bd. 45.

HANCE, R. T. 1917 The fixation of mammalian chromosomes. Anat. Rec., vol. 12.

1917 The diploid chromosomes of the pig (Sus scrofa). Jour. Morph., vol. 30.

1917 The somatic mitosis of the mosquito, Culex pipiens. Jour. Morph., vol. 28.

1918 Variations in somatic chromosomes. Biol. Bull., vol. 35.

HARTMANN, F. A. 1913 Variations in size of chromosomes. Biol. Bull., vol. 24.

HINDERER, H. 1891 Ein Fall von Festhalten der Larvenform bei Frosch lurchen. Blätter f. Aquarien- u. Terrarienkunde, Bd. 2 (quoted from Adler).

JANSSENS, F. A. 1901 La spermatogénèse chez les Tritons. La Cellule, T. 19.
1905 Evolution des auxocytes mâles du Batracoseps attenuatus. La Cellule, T. 22.
1909 La Théorie de la chiasmatypie. La Cellule, T. 25.

JANSSENS, F. A., ET DUMEZ, R. 1903 L'élément nucléinien pendant les cinèsis de maturation des spermatocytes chez Batracoseps attenuatus et Plethodon cinereus. La Cellule, T. 20.

JANSSENS, F. A., ET WILLEMS, J. 1908 La spermatogénèse L'Alytes Obstetricans. La Cellule, T. 25.

JULLIEN 1868 Observations de têtards de Lissotriton punctatus reproduisant l'espèce. Paris. Compt. rend., T. 68.

KINGERY, H. M. 1914 So-called parthenogenesis in the white mouse. Biol. Bull., vol. 27.
1917–18 Oögenesis in the white mouse. Jour. Morph., vol. 30.

KINGSBURY, B. F. 1899 Reducing division in the spermatogenesis of Desmognathus fusca. Zool. Bull., vol. 2.
1901 The spermatogenesis of Desmognathus fusca. Am. Jour. Anat., vol. 1.

KIRKHAM, W. B. 1916 The germ cell cycle in the mouse. Abstract in Anat. Rec., vol. 10.

KUSCHAKEWITSCH, S. 1910 Die Entwicklungsgeschichte der Keimdrüsen von Rana esculenta. Festschr. f. R. Hertwig.

KOLLMANN, J. 1884 Das Überwintern von europäischen Frosch- und Triton- larven und die Umwandlung des mexickanishen Axolotl. Verhandl. d. Naturf. Gesellsch., Basil, 1884.

KAMMERER, P. 1906 Experimentelle Veränderung der Fortpflanzungstätigkeit bei Geburtshelferkröte (Alytes obstetricans) und Laubfrosch (Hyla arborea). Arch. f. Entwicklungsmech., Bd. 22.

KING, H. D. 1907 The spermatogenesis of Bufo lentiginosus. Am. Jour. Anat., vol. 7, no. 3.
1912 Dimorphism in the spermatozoa of Necturus maculosus. Anat. Rec., vol. 16.

KOWALSKI, F. 1904 Reconstitution du noyau et formation des chromosomes dans les cinèses somatiques de la larvae de Salamandre.

KOSTANECKI, V. K. 1892 Über Kernteilungen bei Riesenzellen. Anat. Hefte, Bd. 1.

LAUCHE, A. 1913 Über pluripolare mitosen in Hodenregeneraten von Rana fusca. Arch. f. mikr. Anat., Bd. 82.

MACK, J. B. 1914 A study of the dimensions of the chromosomes of the somatic cells of Amblystoma. Kans. Univ. Science Bull., vol. 9.

MEAD, A. D. 1898 The origin and Behavior of the Centrosomes in the Annelid Egg. Jour. Morph., Vol. XIV, No. 2.

MEVES, F. 1891 Über amitotische Kerntheilung in den Spermatogonien des Salamanders, und das Verhalten des attractionssphären bei derselben. Anat. Anz., bd. 5.

MEVES, F. 1896 Ueber die Entwicklung der männlichen Geschlechtszellen von Salamandra maculosa. Arch. f. mikr. Anat., Bd. 48.
1911 Chromosomenlängen bei Salamandra, nebst Bemerkungen zur Individualitätstheorie der chromosomen. Arch. mikr. Anat., Bd. 77.

METZ, C. W. 1914 A preliminary study of five different types of chromosome groups in the genus Drosophila. Jour. Exp. Zoöl., vol. 17.

McCLUNG, C. E. 1899 A peculiar nuclear element in male reproductive cells of insects. Zool. Bull., no. 2.
1900 The spermatocyte divisions of the Acrididae. Kansas University Quarterly, vol. 9.
1914 A comparative study of the chromosomes in orthopteran spermatogenesis. Jour. Morph., vol. 25.
1917 The multiple chromosomes of Hesperotettix and Mermiria (Orthoptera). Jour. Morph., vol. 29.

MONTGOMERY, T. H. 1903 The heterotypical maturation mitosis in Amphibia and its general significance. Biol. Bull., vol. 4, no. 5.
1911 The spermatogenesis of an Hemipteran, Euschistus. Jour. Morph., vol. 22.

MORGAN, T. H. 1917 Heredity and sex. Columbia University Press.

NOWLIN, NADINE 1908 The chromosome complex of Melanoplus bivitattus Say. Kansas Univ. Science Bull., no. 4.

NUSSBAUM, M. 1880 Zur Differenzierung des Geschlechts im Tierreich. Arch. f. Mikr. Anat., Bd. 18.

PAYNE, F. 1914 Chromosomal variations and the formation of the first spermatocyte chromosomes in the European earwig, Forficula sp. Jour. Morph., vol. 25.

PARMENTER, C. L. 1919 Chromosome number and pairs in the somatic mitosis of Amblystoma tigrinum. Jour. Morph., vol. 33, no. 1.
1920 The chromosemes of parthenogenetic frogs. Jour. Gen. Physiol., vol. 11, no. 3.

PLOUGH, H. H. 1917 The effect of temperature on linkage in the second chromosome of Drosophila. Proc. Nat. Acad. Science, vol. 3.

RAWITZ, B. 1895 Centrosoma und Attraktionssphäre in der ruhenden Zelle des Salamanderhodens. Arch. f. mikr. Anat., Bd. 44.

ROBERTSON, W. R. B. 1915 Chromosome studies. III. Inequalities and deficiencies in homologous chromosomes. Jour. Morph., vol. 26.
1916 Chromosome studies. I. Taxonomic relations shown in the chromosomes of Tettigidae and Acrididae: V-shaped chromosomes and their significance in the Acrididae, Locustidae and Gryllida: Chromosomes and variation. Jour. Morph., vol. 27.

RUBASCHKIN, W. 1912 Zur Lehre von der Keimbahn bei Säugetieren. Anat. Hefte, Bd. 46.

SCHREINER, A. AND K. E. 1906 a Neue Studien uber die Chromatinreifung der Geschlechtszellen. I. Die Reifung der männlichen Geschlechtszellen von Tomopterus onisciformis. Arch. de Biol., T. 22.
1906 b Ibid. II. Reifung der männlichen Geschlechtszellen von Salamandra maculosa, Spinax niger and Myxine glutinosa. Ibid.

SNOOK, H. J., AND LONG, J. A. 1914 Parasynaptic stages in the testis of Aneides lugubris (Hallowell). University California, pub., vol. 15.

STEVENS, N. M. 1908 A study of the germ cells of certain Diptera, with reference to the heterochromosomes and the phenomena of synapsis. Jour. Exp. Zoöl., vol. 5.

SWINGLE, W. W. 1917 The accessory chromosome in a frog possessing marked hermaphroditic tendencies. Biol. Bull., vol. 33.
1918 The effects of inanition upon the development of the germ glands and germ cells of frog larvae. Jour. Exp. Zoöl., vol. 24, no. 3.
1918 The acceleration of metamorphosis in frog larvae by thyroid feeding and the effects upon the alimentary tract and sex glands. Jour. Exp. Zoöl., Vol. 24, no. 3.
1919 Studies on the relation of iodine to the thyroid. I and II. Jour. Exp. Zoöl., vol. 27, no. 3.
1919 Iodine and the thyroid. III and IV. Jour. Gen. Physiol., vols. 2 and 3.
1920 Homoplastic thyroidal transplants and the metamorphosis of Rana catesbeiana. Anat. Assoc. Abstracts.
1920 Neoteny and the sexual problem. Amer. Nat., July–August.

FISCHER-SIGWART, H. 1896 Die Fortpflanzung und die Entwicklung der Larven vom Molge vulgaris. Das Überwintern der Larven. Der Zool. Garten, No. 37.

SHUFELDT, R. W. 1885 Mexican axolotl and its susceptibility to transformation. Science, vol. 6.

VON RATH, O. 1893 Ueber die Bedeutung der amitotischen Kerntheilung im Hoden. Zool. Anz., Bd. 14.
1893 Beiträge zur Spermatogenese von Salamandra. Zeitschr. f. Wiss. Zool., Bd. 57.

VON WINIWARTER ET SAINMONT 1908–1909 Nouvelles recherches sur l'ovogénèse et l'organogénèse de l'ovaire dans mammifères (Chat). Arch. de Biol., T. 24.

VEJDÖVSKY, F. 1911–12 Zum problem der Vererbungsträger. Böhm. Gesell. Wiss., Prag.

WENRICH, D. H. 1917 Synapsis and chromosome organization in Chorthippus (Stenobothrus) curtipinnis and Trimerotropos suffusa (Orthoptera). Jour. Morph., vol. 29.
1916 The spermatogenesis of Phrynotettix magnus with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zoöl. Harvard, vol. 60.

WILSON, E. B. 1906 Studies on chromosomes. III. The sexual differences of the chromosomes in Hemiptera, etc. Jour. Exp. Zoöl., vol. 3.
1909 Studies on chromosomes. V. The chromosomes of Metapodius, etc. Ibid., vol. 6.
1910 Studies on chromosomes. VI. A new type of chromosome combination in Metapodius. Ibid., vol. 9.
1911 Studies on chromosomes. VII. A review of the chromosomes of Nezara, etc. Jour. Morph., vol. 22.
1912 Studies on chromosomes. VIII. Observations on the maturation phenomena in certain Hemiptera, etc. Jour. Exp. Zoöl., vol. 13.

WINIWARTER, H. VON 1900 Recherches sur l'ovogénèse et l'organogénèse de l'ovaire des mammifères (Lapin et Homme). Arch. de Biol., T. 17.

Witschi, Emil 1914 Experimentelle Untersuchungen uber die Entwicklungs-
geschichte der Keimdrüsen von Rana temporaria. Arch. f. mikr.
Anat., Bd. 85.

Wright, A. H. 1914 North American Anura. Life-histories of the Anura of
Ithaca, New York. Publ. Carnegie Institute, Washington.

Walterstorff, W. 1896 Über die Neotenie der Batrachier. Der Zool. Gar-
ten, no. 37.

Wichand, B. 1906 Über Neotenie bei Tritonen. Blätter für Aquarien- und
Terrarienkunde (quoted from Adler, Leo).

Woolsey, C. I. 1915 Linkage of chromosomes correlated with relation in num-
bers among the species of a genus, also within a species of Locustidae.
Biol. Bull., vol. 28.

EXPLANATION OF PLATES

All drawings were outlined with camera lucida; 2-mm. oil-immersion objective used, ocular 12; hence are of the same magnification. Plates 1, 2, and 3 have been reduced one-third.

PLATE 1

EXPLANATION OF FIGURES

1 Primary spermatogonium surrounded by follicle. Note polymorphic nucleus, i.e., chromosomal vesicles. From first-year larvae.

2 Diagram of early division prophase of primary spermatogonium. The chromosomes appear in vesicles which make up the entire nucleus and give it the lobulated appearance.

3 Diagram of later prophase. Chromosome vesicles have disappeared.

4 and 6 Equatorial plates showing twenty-eight chromosomes. Note Chromosome pair marked A. The peculiarity of an end-piece attached by a non-staining area is constant.

5 Odd type of spermatogonial prophase chromosomes may appear in best fixed material. Chromosomes appear as solid balls. Abnormal cell evidently degenerating.

7 Odd cell division. Spindle oriented in short axis of cell.

8 Resting nucleus after last spermatogonial telophase. Note the chromatin blocks and linin fibrils. Larvae 45 mm. total length.

9 Preleptotene stage showing resolution of the chromatin blocks into fine threads. Larvae 40 mm. total length.

10 Large cell with leptotene threads. Note the chromoplasts with attached fibrils.

11 Amphitene nucleus. Pachytene loops at proximal pole, unpaired leptotene filaments at distal pole. This cell illustrates the formation of the pachytene bouquet. See plate 5, fig. 39. Larva 45 mm. total length.

12 Isolated pachytene threads showing unpaired leptotene filaments at distal ends. Compare with figures 36 to 39, plate 5.
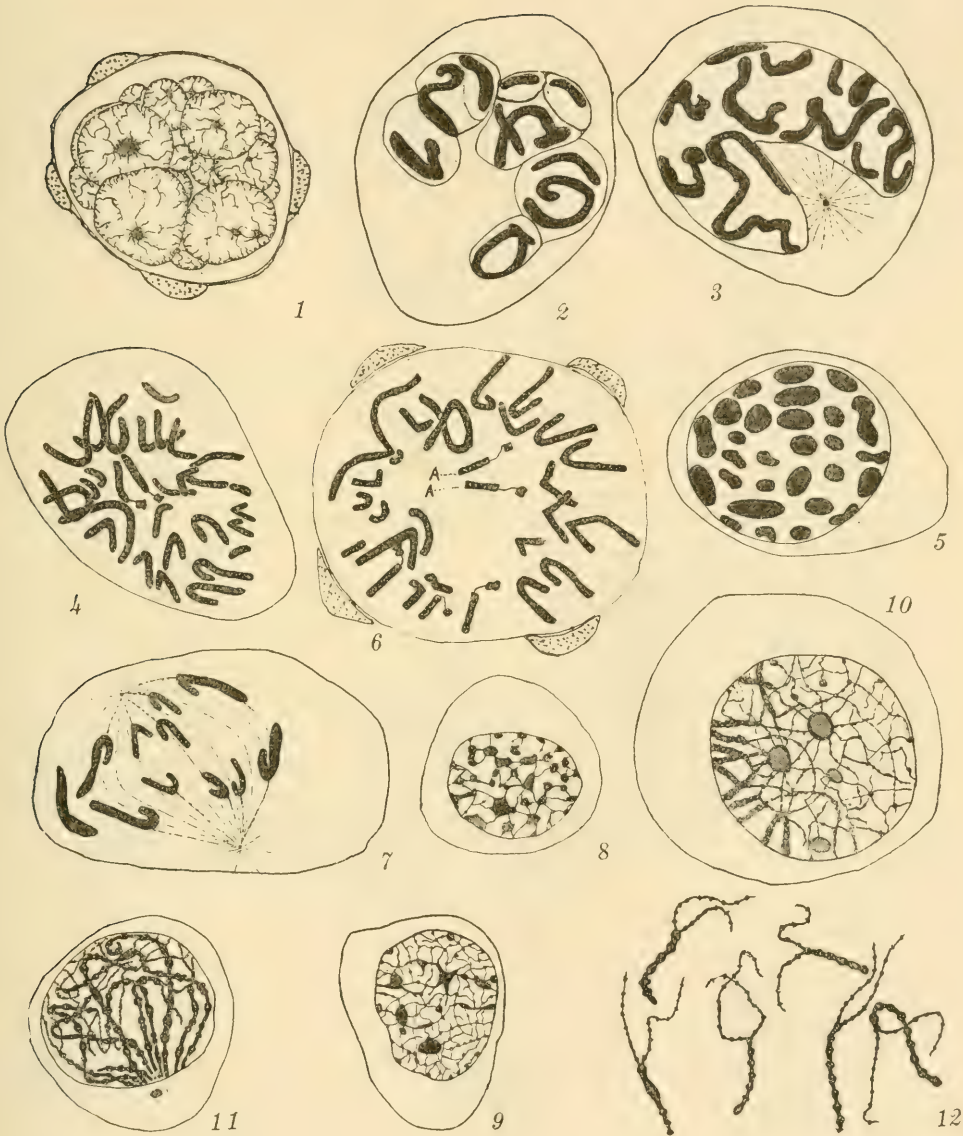
13 Amphitene nucleus. Larva 45 mm.

14 Diplotene stage showing splitting of the thick pachytene threads and their separation in the middle. Note attachment at synaptic ends.

15 Diplotene nucleus. Compare with figures in plates 5 and 6. Larvae 45 to 60 mm.

16 Diplotene nucleus showing early formation of tetrads. Larvae 45 to 60 mm. No evidence of crossing-over.

17 Condensation of the type of tetrad shown in figure 16. Note the woolly appearance. Larva 74 mm.

18 First spermatocyte tetrads and tripolar spindle. Fourteen tetrads present. First-year tadpole. See figure 79.

19 Spermatocyte with eighteen tetrads. Very unusual condition. Larvae 80 mm. total length.

20 Tetrads of larvae of first year. See plates 9 to 11. X in figure 20 indicates persisting karysomal structure of unknown origin and fate.

21 Spermatocyte with multiple asters. First-year larvae 40 to 50 mm. total length.

22 Giant spermatid-like body resulting from degeneration of larval spermatocytes like those shown in figures 106 and 108. See also plate 13.

23 Different type of spermatid-like body. The black masses represent the ring tetrads which have run together. See plate 13 also. Larva 80 mm.

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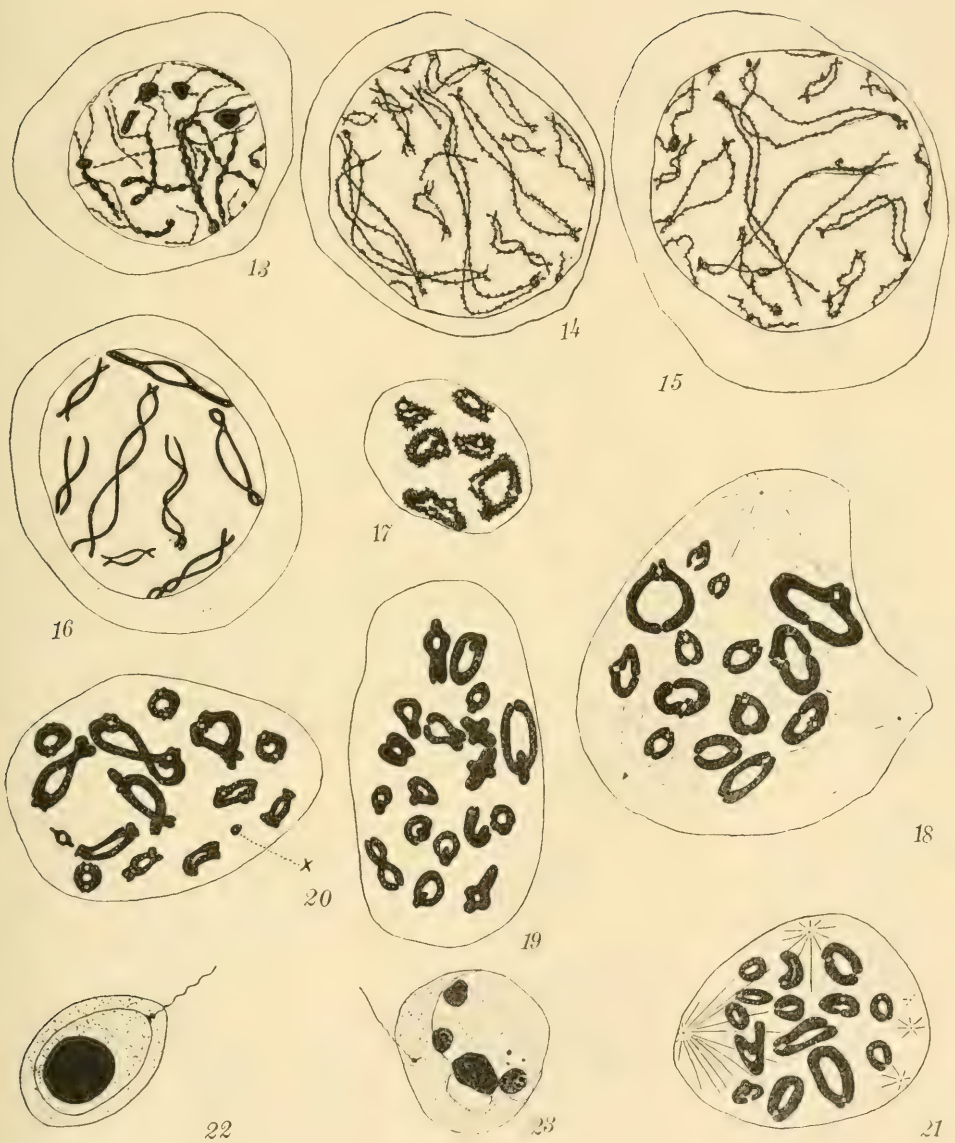
PLATE 3

Fig. 24 and 25   Large larval spermatocyte.   Cell cut.   Both figures are of one cell.   Note the body resembling a yolk nucleus in figure 25.   Larva 100 mm.

26 and 27   Both figures of same cell.   Note rod-shaped tetrad in figure 26. Unusual condition.   Larva 80 mm.

28   Giant spermatocyte of first-year larva.   Note rod tetrad and extreme nuclear size.   This cell shown in photographs 64 and 67.   Larva 80 mm.

29 and 30   Sections of the same cell.   Note the Y-shaped and cross-shaped tetrads.   Cell of unusual size.   Note similarity of tetrads to those of urodeles. Same cell shown in photographs 87 and 88.   Larva 100 mm.

31 and 32   Sections of same cell.   Note the rod-shaped and cross tetrads. Cell of unusual size.   Figure 114 is a photograph of a portion of this cell.   Larva 95 mm.

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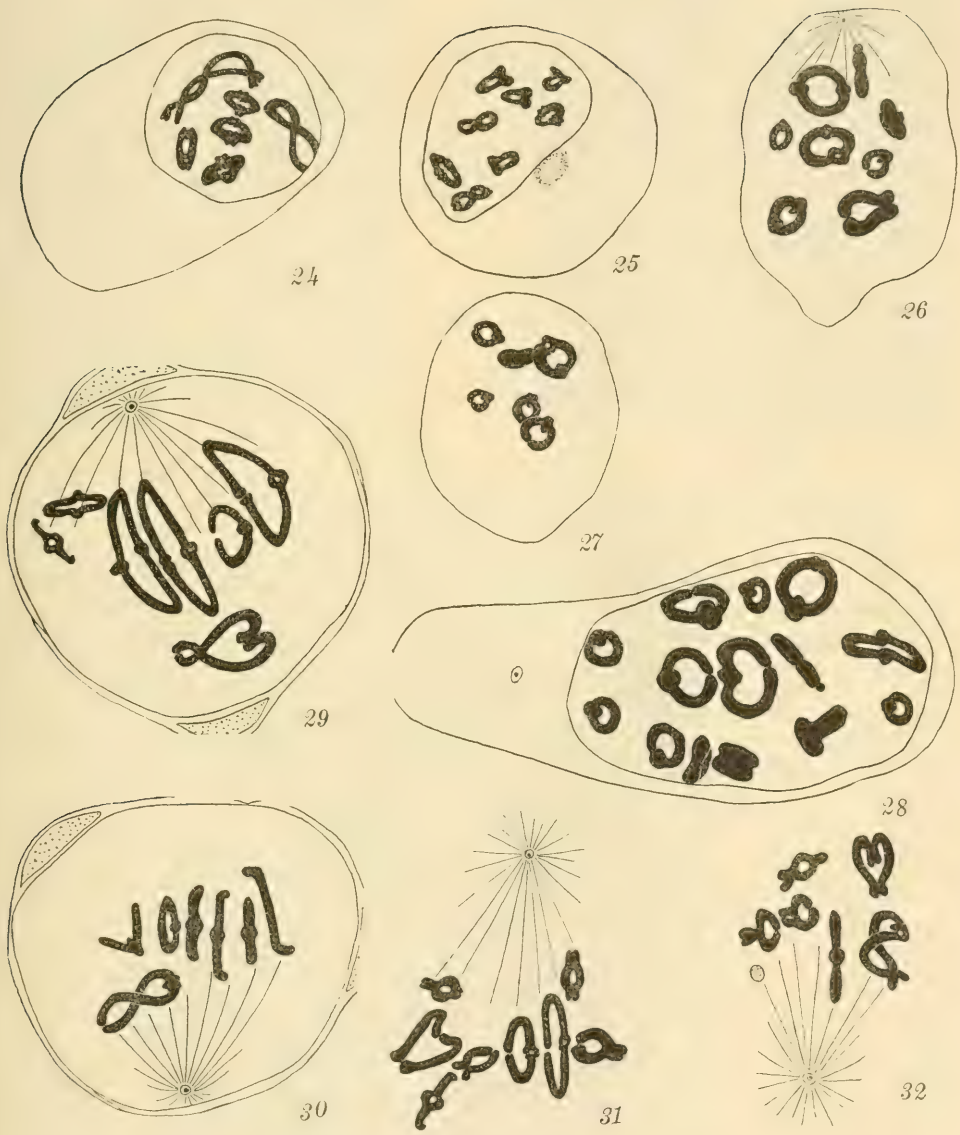
Microphotographs on this plate made at magnification of 50 diameters. No reduction.

33 Transverse section through male gonad shown in text figure 1, a. Note the very large secondary genital cavity surrounded by double layer of germ cells. Animal 40 to 50 mm. total length. Maturation of the germ cells begins in many instances in undifferentiated glands of this type.

34 Cross-section through male gonad of first-year tadpole (text fig. 1, b). The large cavity is disappearing, owing to rapid proliferation of germ cells. The cavity is lined by non-sexual mesodermal cells which have migrated in from the mesonephros. Practically all the germ cells in this type of larvae gonad are maturating. Animal 70 to 95 mm. total length.

35 Section through male gonad of second-year tadpole approaching metamorphosis (text fig. 1, C). The cavity has disappeared. Note the testicular ampullae. The second sexual cycle occurs in this type of testis. Animals 120 to 150 mm. total length.

Microphotographs made at a magnification of 1500 diameters. No reduction.

36   Amphitene nuclei. Note the thick pachytene thread at one pole and the fine leptotene filaments at the opposite pole. Synapsis occurs at this stage.

37 and 38   Amphitene nuclei from first-year larvae. No effort has been made to show the cytoplasm.

39   Pachytene nuclei. Synapsis complete. The threads appear single.

40   Transition stage from pachytene to diplotene nuclei. The thick pachytene threads of figure 39 are in figure 40 splittion into two thin threads.

41   Diplotene stages showing the disjunction of the leptotene threads paired in figure 39. Note at X the split in the thick thread. This is the primary longitudinal split.

42   Diplotene stage showing splitting of the thick pachytene threads.

43 and 44   Diplotene stages showing the figure-8 configuration of the splitting threads.
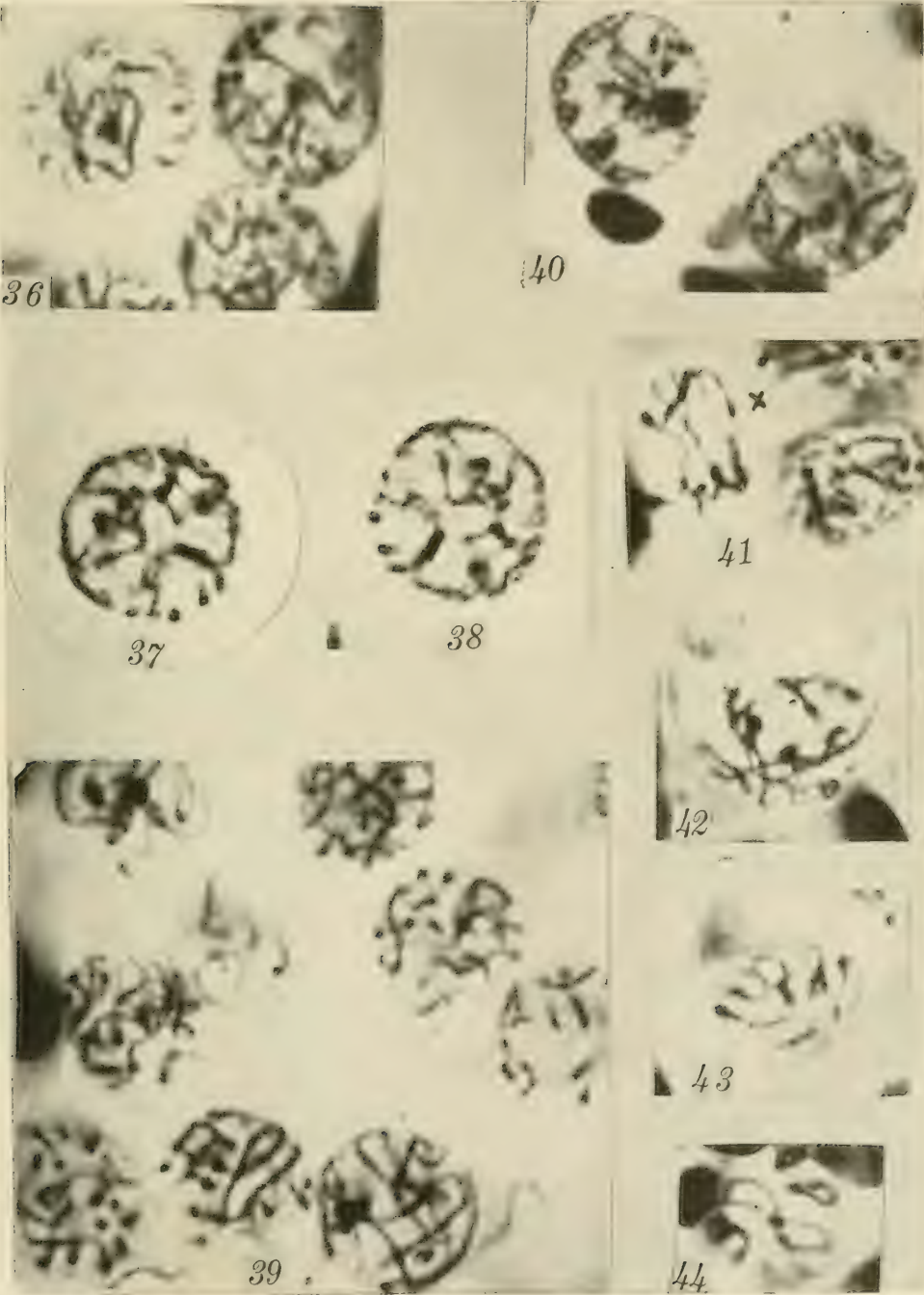
45   The large cell near the upper central edge of the photograph is a stage in middle diplotene, showing the disjunction of the homologous chromosomes in the shape of fine threads. The threads, it will be observed, are united at their ends. Several pachytene nuclei are visible at the lower left-hand corner. In the lower right-hand corner are shown portions of three ring tetrads.

46   Gigantic diplotene cell. This type of cell is very common; the size of the nuclei is remarkable in some instances. Note the split threads extending across the nucleus, but united at the ends by two dark staining knobs.

47   Portion of a diplotene nucleus together with two early preleptotene nuclei. Note the size difference.

48   Diplotene nucleus.

49   Part of a diplotene nucleus, showing splitting of the threads.

50 and 51   Large diplotene nuclei. Larva of 80 mm.

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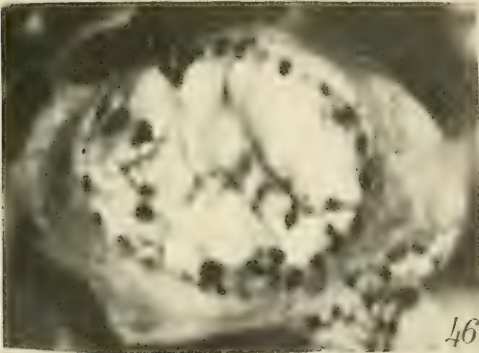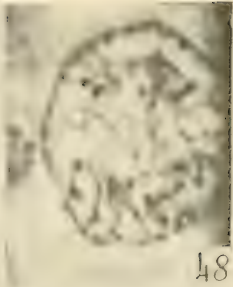
52 to 54 Portions of large diplotene nuclei, showing condensation and great thickening of the threads to form the tetrads. Note the size of the chromosomes. All are cut in these photographs; the size of the nuclei is so great that each one of these cells is cut into three parts when sectioned at a thickness of 8 to 10μ. In figure 54 note that the condensing chromosomes are split again—equational split.

55 and 56 Portions of large cells in diakinesis. The ring tetrads are shown.

57 Large diplotene nuclei. Nucleus almost completely fills cytoplasm.
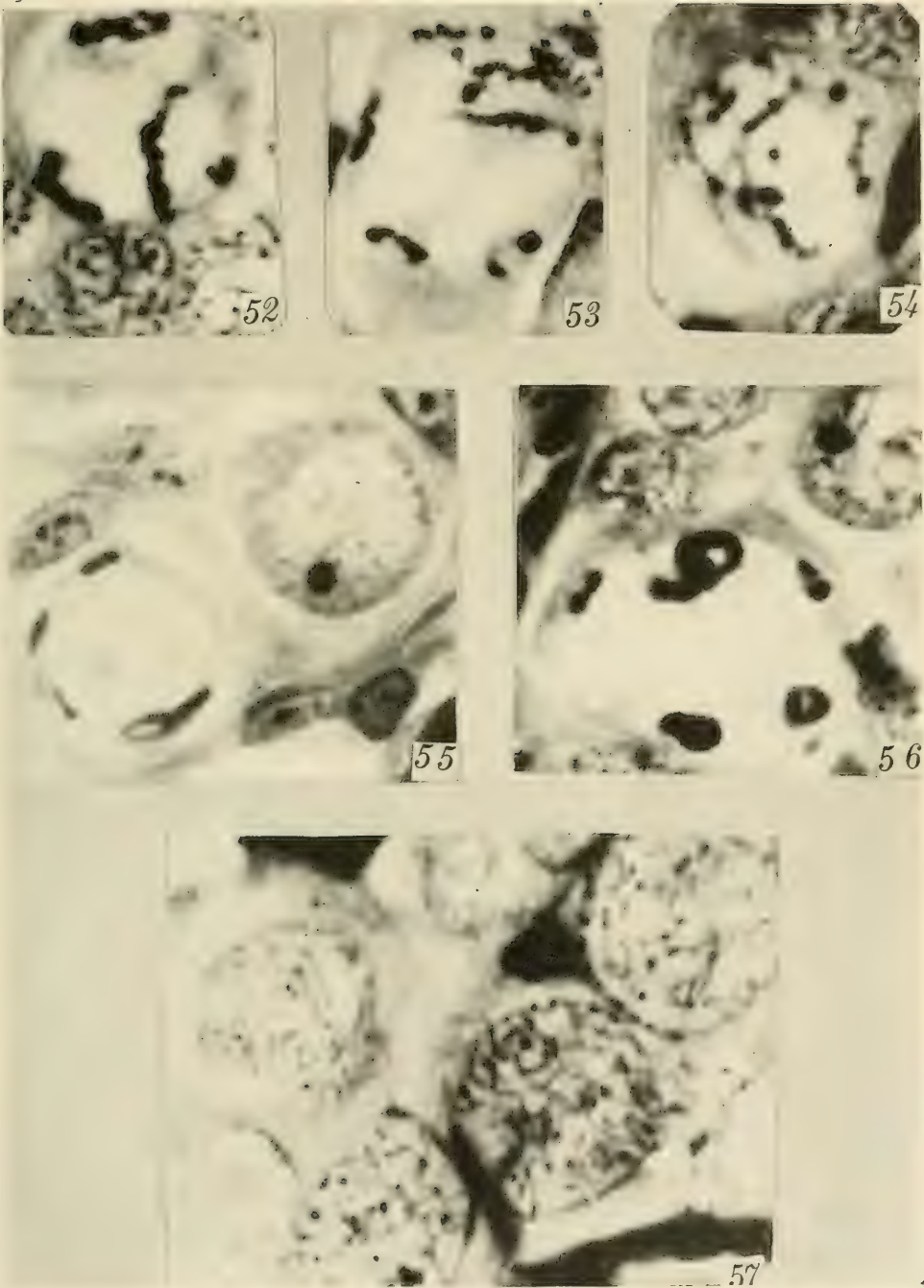
58 Portion of large nucleus in diakinesis stage. Practically no cytoplasm surrounding nucleus.

59 to 63 Smaller cells in diakinesis. Note figure-8 chromosomes.

62 Portion of large nucleus in diakinesis. Note the extraordinary large figure 8 in this cell.

64 . Portion of large spermatocyte, showing ring tetrads.

65 and 66 Portions of spermatocytes showing chromosomes.

67 Same cell as figure 64 photographed at a different focus. Note follicle cells and nuclear size. Compare these cells with those of plate 15.
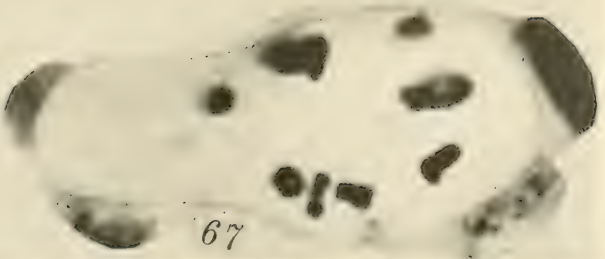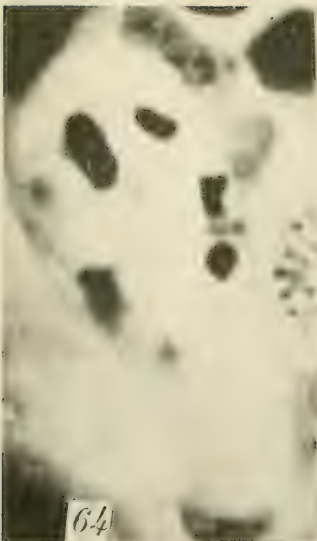
68   Portion of large spermatocyte nucleus.

67 to 76   Ring tetrads of first-year larval spermatocytes.   Note the large open rings and especially the size of certain of the tratrads.   In adult frogs the tetrads on the spindle are not in the form of open rings of this type, but resemble dumbbells and are very much smaller in size.   Compare with plate 15.
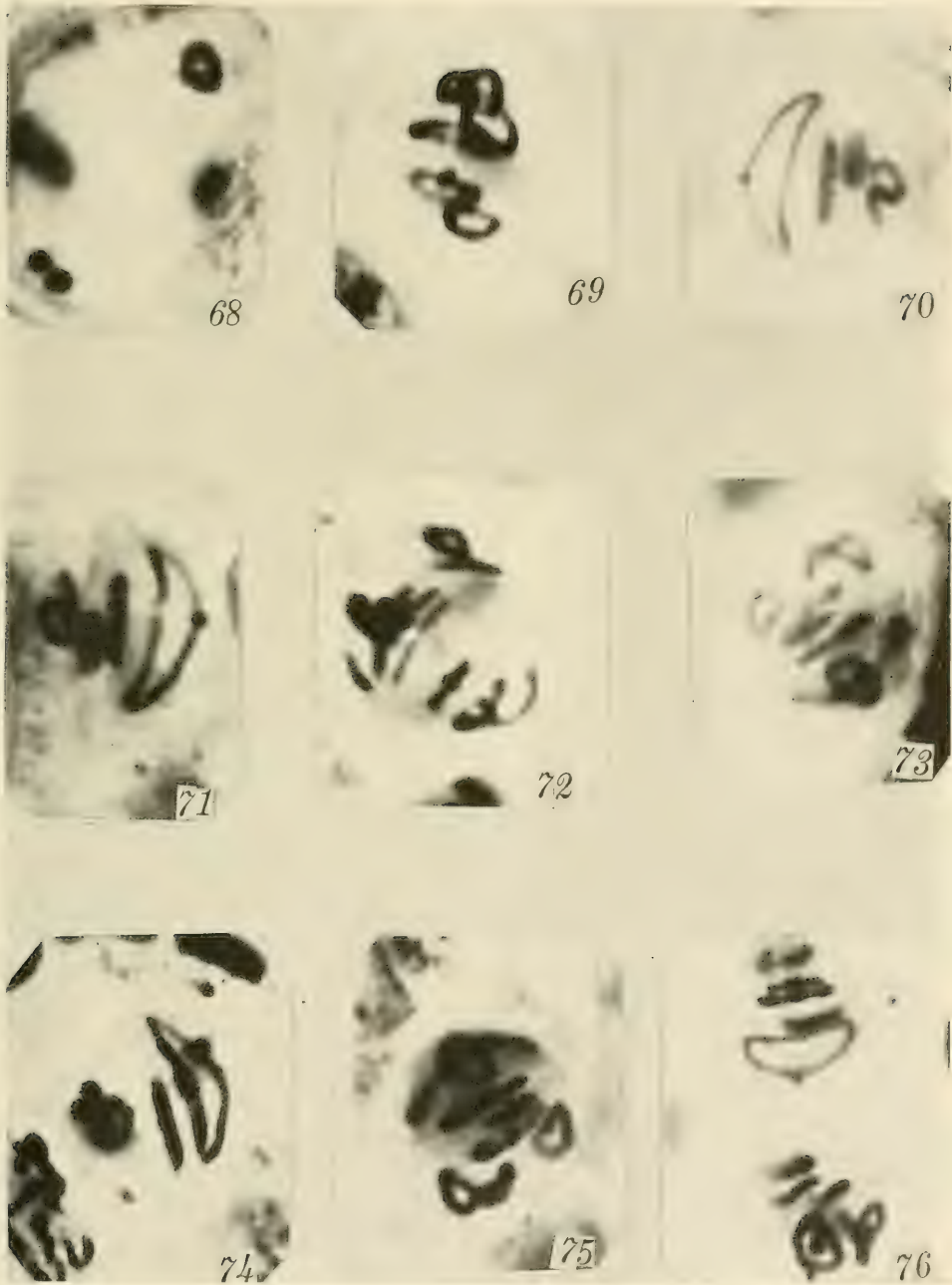
77 Large first spermatocyte in act of division. Note the size of the tetrads and spindle. These cells degenerate in the act of dividing at this stage.

78 to 80 Large spermatocytes of first-year larvae in act of division. Figure 79 shows a tripolar spindle and irregular arrangement of the tetrads.

81 and 82 Larval spermatocytes. Figure 82 is of a cell in process of degeneration. The tetrads have lost their annular appearance.

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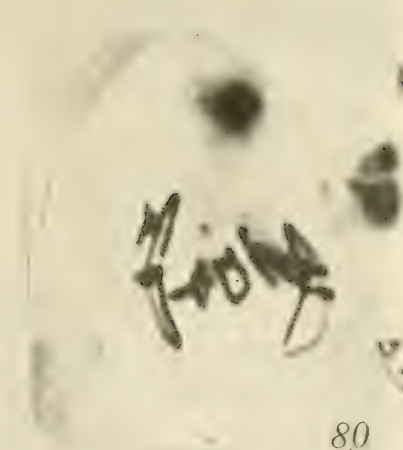
83   Giant spermatocyte in division.   Note size of the cell and its follicle.

84   A portion of large spermatocyte preparing for division.   All such cells degenerate before completing the process.

85 to 88   Large larval spermatocytes preparing for division.   All degenerate before completing the process.

89 and 90   Are isolated tetrads of larval germ cells in similar stages of development.   Note variation in size of the tetrads in these cells.   Figure 90 is from animal in second year.   It is rare to find this type of tetrad in second-year larvae. Compare with plate 15.
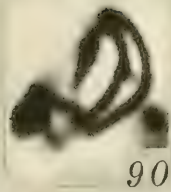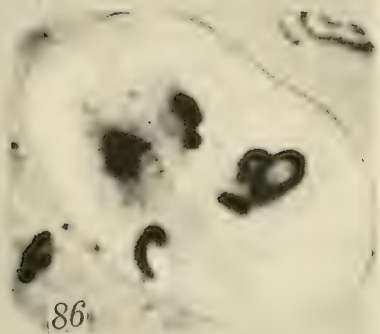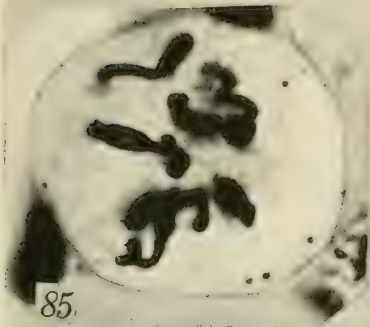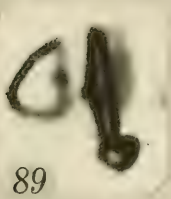
91 to 94   Larval spermatocytes dividing.

95   Single large ring tetrad.   Note the size and the lugs marking the synaptic ends of the homologous chromosomes.

96 to 99   Larval spermatocytes dividing abnormally.   Note in figure 97 the great size and thickness of the central chromosome.   This tetrad is cut in half longitudinally.

100 and 101   Abnormal spermatocyte.   Many tiny asters scattered through the cytoplasm.

102   Large ring tetrad from second-year larvae.   Just beneath is a small germ cell of the second year; note the size difference.   The spermatocyte in which this tetrad was photographed is not shown here and was in early stages of degeneration.   This type of tetrad rare in second-year tadpoles and found only in degenerating cells, which persist from first sexual cycle.

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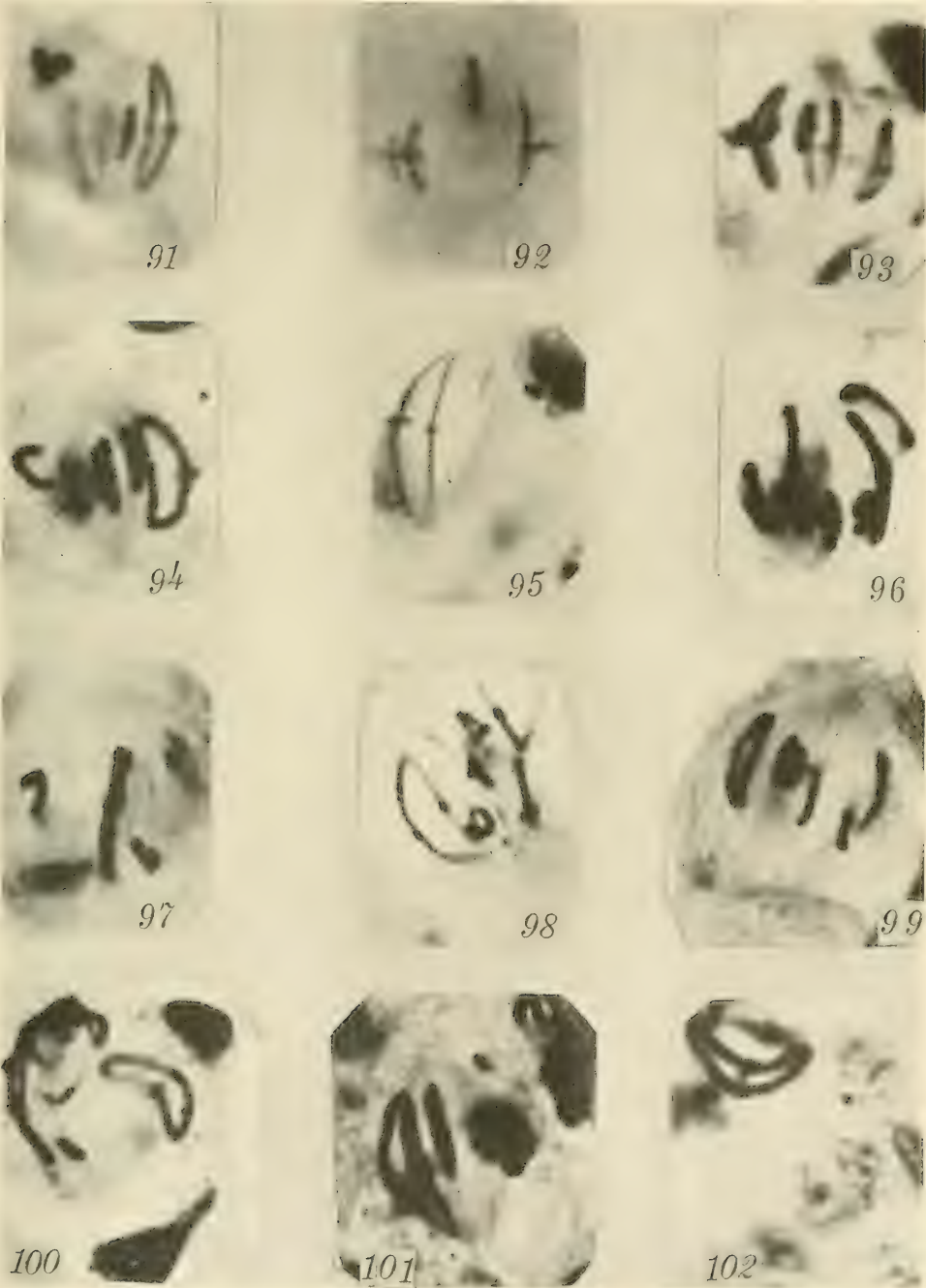
PLATE 13

103   Larval spermatocyte in early stages of degeneration.   Tetrads condensing or running together.

104 and 105. Further stages in spermatocyte degeneration.

106 to 108   End stages of degeneration of larval spermatocytes.   The type of cell depicted in figures 106 and 108 sometimes show long axial filaments as outgrowths of the centrosome.   The black balls are the remains of the tetrads.

109 and 110   Stages in the degeneration of the larval spermatocytes of the first sexual cycle.   Note in figure 109 the condensed group of vacuoles in the large clear area.   The clear area represents the original size of the cell; the vacuoles are the remains of the tetrads that went to pieces in situ on the first maturation spindle.
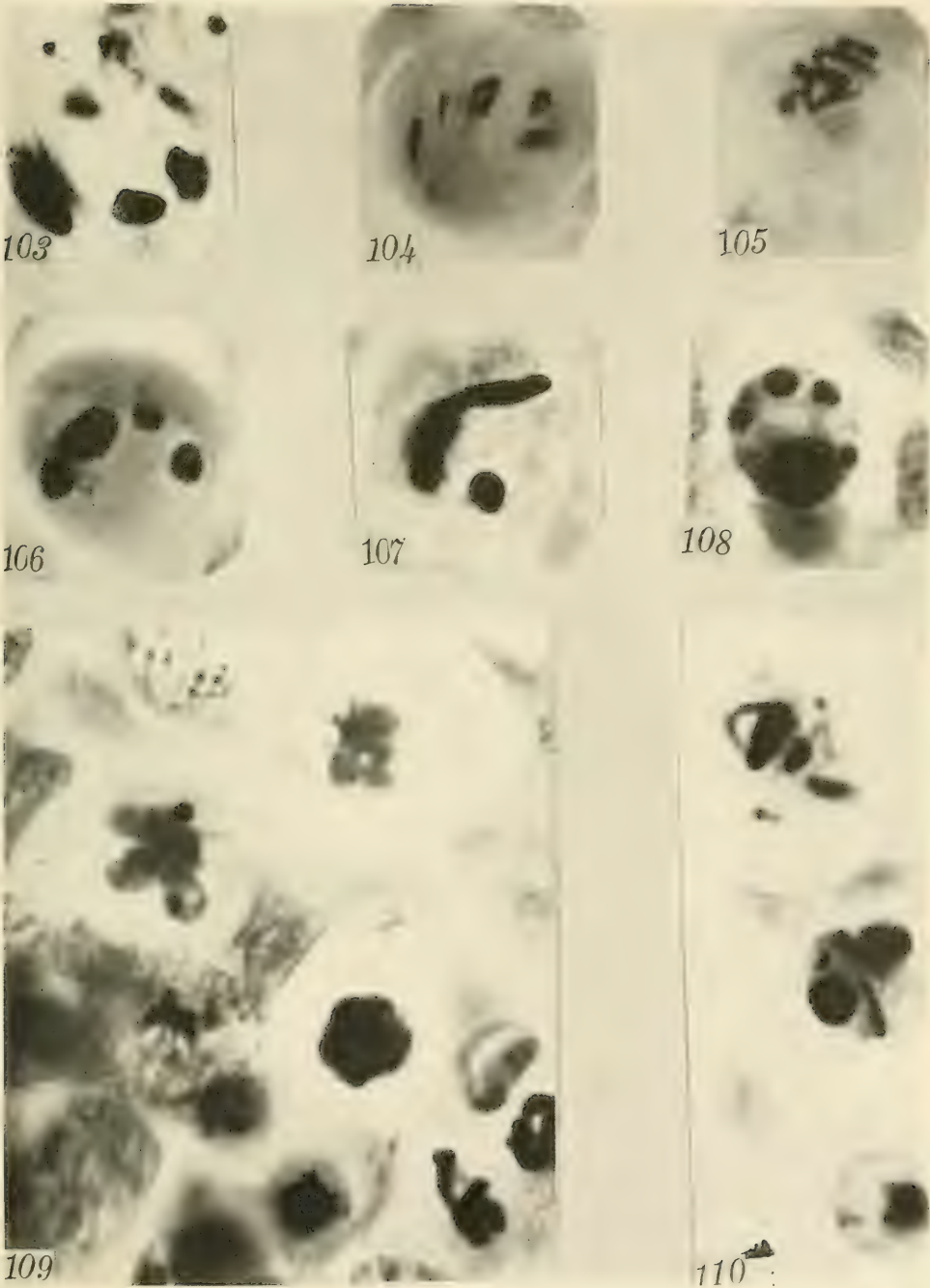
111   So-called 'oocyte' in larval testis.   The character and history of these cells will be discussed in another communication.

112   Giant larval spermatocyte, showing size of the spindle.

113   Giant spermatogonium from testis of first-year larvae.

114   Large spermatocyte dividing.   Only half of the cell is shown.   Note the extreme length of spindle.

115   Spermatogonium showing polymorphic nucleus.   At left of picture is an abnormal spermatocyte division.   The chromosomes are torn to pieces by polyasters in the cytoplasm.
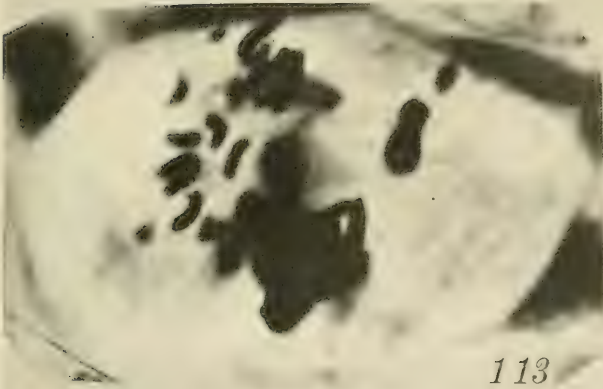
116   Gigantic spermatocyte dividing, showing tripolar spindle.   Note distribution of chromosomes on the two spindles.
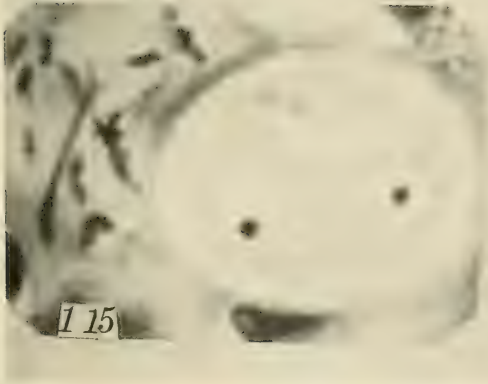
PLATE 15

117 Primordial spermatogonium with large vesicular nucleus. This type of cell, in very small numbers, persists unchanged through the first-year maturation cycle of the larvae, and by repeated divisions probably contributes the cells of the second sexual cycle of the larvae.

118 and 119 Small germ cells of second-year tadpoles preparing for the second sexual cycle. Compare these cells with figure 117. Both types of cell are found in the same gonad. The smaller elements appear to originate in part from transformed mesothelial cells (?).
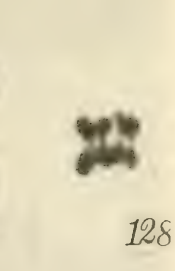
120 to 123 First spermatocyte prophases. Note the small size of the cells and tetrads. Compare with figures 112 or 114 or any cell on plates 7 to 13.

124 to 128 First spermatocyte divisions of second larval sexual cycle, or newly metamorphosed frogs. Note extremely small size of cells and tetrads. Compare with plates 7 to 13. Clearness of detail of the chromosomes has been sacrificed by overdevelopment to show the spindles and cell outlines.

129 and 130 Spermatids of second sexual cycle.

131 Spermatozoa of second larval sexual cycle. These are sometimes formed in large numbers in the larvae, but are more numerous shortly after metamorphosis.

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Resumen por los autores, John A. Detlefsen y Elmer Roberts,
Illinois Agricultural Experiment Station.
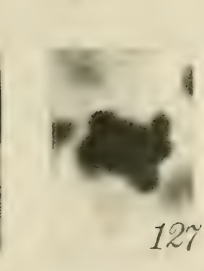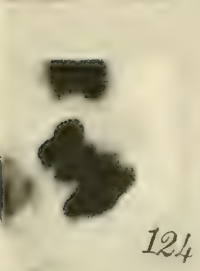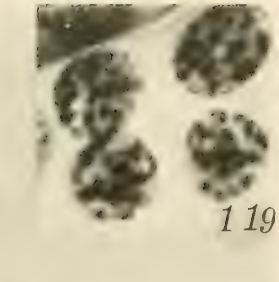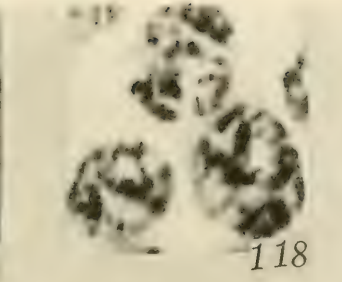
Estudios sobre el crossing over.

I. El efecto de la selección sobre los valores del crossing over.

El tanto por ciento de cross overs de la combinación de ojos
blancos y ala en miniatura de Drosophila melanogaster es próxi-
mamente 33, y la "distancia de mapa" hallada por Morgan y
Bridges es próximamente 36. La selección de hembras que
presentaban valores bajos en el crossing over redujo el tanto
por ciento de estos últimos casi a O en la serie A y su derivada
serie A'. Estas dos series reprodujeron este valor reducido del
crossing over durante tres y nueve generaciones, respectiva-
mente. En otra serie independiente B, el valor del crossing over
se redujo a 5 o 6 por ciento en 28 generaciones y el tronco selec-
cionado ha continuado produciendo dicho crossing over, sin
más selección, durante 22 generaciones. La serie C, con crossing
over alto, no produjo por selección un aumento en los valores de
crossing over, pero produjo en la F, nueve pares que sumaban
26 cross overs; 1055 individuos—2.46 por ciento de crossing over.

El crossing over es por consiguiente muy variable y manifiesta
los efectos de la selección. La selección de valores bajos ha
eliminado prácticamente el crossing over en las series A y A',
y le ha reducido considerablemente en al serie B. La selección
de un valor elevado no ha aumentado los valores de crossing
over en la serie C, pero probablemente ha producido más crossing
over doble en algunas hembras, que resulta en una disminución
del valor de dicho proceso con relación a los dos genes escojidos.
Los autores proponen varias hipótesis, pero parece sumamente
probable que los factores múltiples regulan o por lo menos
influyen considerablemente sobre el crossing over. Si esta
explicación es correcta, debemos modificar nuestra opinión sobre
el crossing over en relación a las distancias que separan a los
genes.

STUDIES ON CROSSING OVER

I. THE EFFECT OF SELECTION ON CROSSOVER VALUES[1]

J. A DETLEFSEN AND E. ROBERTS[2]

TWO TEXT FIGURES

CONTENTS

INTRODUCTION

The experiments described in this paper were undertaken in an effort to answer the question: can the percentage of crossing over be modified by selection. The significance of an answer (either affirmative or negative) and its relation to our present concepts on crossing over, locus, chromosome mapping, etc., were apparent and seemed to justify the time and attention necessary to carry these investigations over so long a period. They were planned and begun in February, 1916, and have involved the classification of over 300,000 individuals. Had the results been negative, the experiment would have been

[1] Paper no. 12, from the Laboratory of Genetics, Illinois Agricultural Experiment Station.

[2] We wish to give credit to the following graduate students for aid in these investigations: Mr. A. T. Fishman carried series A for three generations; Mr. L. E. Thorne carried series C for seven generations. The war called both men from their work. The late Prof. B. O. Severson carried series B from the beginning to the F_{14} generation. In the death of Professor Severson both genetics and scientific agriculture have lost a capable and enthusiastic student and investigator.

dropped much earlier, but the effects of selection were conspicuous and prompted us to carry the experiments through to their logical end.

Whenever one crosses an individual with the linked factors, AB, to the double recessive, ab, then the heterozygote, ABab, will form four sorts of gametes: AB + ab, the parental types, and aB + Ab, the recombinations or crossovers. The relative frequencies of these gametes will depend upon the distance between the loci for A and B, at least according to the commonly accepted hypothesis. If a distance on the chromosome, which gives 1 per cent of crossovers is adopted as an arbitrary unit, then the distance between genes on a chromosome may be determined in terms of this arbitrary unit, and the map of a chromosome may be plotted, as has been done by investigators working with Drosophila. Repeated trials using large numbers, with comparable stocks and controlled environmental conditions, have shown that the ratio of crossovers to total gametes is uniform enough to suggest that the distance between two genes is fairly constant. However, the phenomenon of crossing over is not as simple as was first supposed, for a number of genetic and environmental influences have been found to affect crossing over markedly, at least in Drosophila melanogaster. Bridges ('15) stated that crossing over varied with age, for second broods showed a rather consistent decrease. Plough ('17) found that low and high temperatures (below 17.5°C., and above 28°C.) increase the amount of crossing over. Sturtevant ('19) found in the second chromosome of Drosophila one gene to the left of purple and one to the right, both of which lower the percentage of crossing over in that portion of the chromosome in which they lie. He also found a similar factor in the third chromosome. Furthermore, an incompletely investigated case disclosed a dominant third chromosome gene which increased the amount of crossing over between purple and curved in the second chromosome. Gowen ('19) measured the amount of variability shown in a population of 240 Drosophila females with respect to crossing over between fixed points in the third chromosomes and found a very high degree of variability. His data show that

a change in genes between two or more fixed points may be accompanied by a slight disturbance of the crossing-over ratios between these fixed points. Sex, to be sure, has a striking effect on crossing over, for the male Drosophila does not show this phenomenon even in the autosomes.

Whenever one observes a large number of Drosophila females of the generalized zygotic formula $\dfrac{A}{a} \quad \dfrac{B}{b}$, it is common to find much variability with respect to the amount of crossing over, even though the cultures are kept at the usual normal temperature and no striking genetic modifiers of crossing over are known to exist. Just what this variability is due to is not known. Some of it may represent fluctuations of sampling and some of it may be due to age, but very frequently the deviations are so wide as to arouse a suspicion that hitherto unknown causes may be effective. If this variability is due, at least in part, to genetic causes, then selection should have an effect, particularly if environmental fluctuations do not mask or obliterate the effect of genetic modifiers. It was with this thought in mind that the senior writer began to select for high and low crossover values.

MATERIALS AND METHODS

The selection experiments consisted of four series:

Series A, low selection;

Series A', derived from series A in F_7;

Series B, low selection, a second experiment duplicating series A;

Series C, high selection.

Each series began with a single white-eyed miniature-winged female mated to a wild red long male. These strains were chosen because the characters are easily recognized, show little or no variability, and have at least fair viability. To classify any female with respect to her 'crossover capacity' requires the classification of all the progeny which we can obtain from her. Thus in F_6, series A (table 1), we classified 8660 offspring to obtain the necessary data on fifty-six F_5 females for the purpose of selection. In the usual selection experiment, individuals are

chosen on the basis of external characters which can be determined by direct observation. To select for high and low crossover values is rather more tedious and time exacting because the individual cannot be classified directly with respect to its crossover potentiality. Its character is disclosed only after obtaining a reasonably large progeny. Characters which could be recognized easily and classified rapidly and accurately were indispensable. The two allelomorphic pairs, white eye vs. red eye, and miniature wing vs. long wing seemed to fulfill these conditions, and they have the added advantage of giving a large, initial, normal percentage of crossovers (about 33 per cent), which means that variations are thus more readily detected.

The procedure followed in the low-selection series A is typical of all the series and can be taken as a sample. A single white miniature female mated to a red long male gave F_1 white miniature males, $\underline{w \qquad m}$, and long red females $\dfrac{w}{W} \quad \dfrac{m}{M'}$ the latter being double heterozygotes. The F_1 sibs were mated in pairs in 8-drachm homeopathic vials, and the pairs were removed to new vials about every three days. The culture methods were those commonly used with Drosophila. The F_2 offspring from each vial were classified daily until a fair sample of each F_1 female's 'crossover capacity' was obtained. As expected, the offspring were of four kinds: the parental types, red long, and white minature and the crossovers red miniature and white long. It was impossible to anticipate which F_1 female was going to be selected because of her low crossover ratio determined by a reasonably large progeny, and it was likewise virtually impossible to continue mating in pairs the sibs from each F_1 female until we could find out which line was going to be used to continue the selection. Thus in F_1 series A, low selection (table 1), there were twenty-eight pairs of F_1 individuals, several of which appeared to be promising material, but we eventually chose pair 15, which gave $21:98 = 21.43$ per cent.[3] By the time we

[3] In giving crossover values we shall put the data in the following order throughout this paper: crossovers: total-per cent of crossing over. The classes are always the same and repetition can thus be avoided.

were in position to know that pair 15 would be selected, practically all of its F_2 offspring had emerged. Therefore it seemed expedient to mate en masse the F_2 offspring (i.e., red long females $\frac{w \quad m}{W \quad M}$, miniature white males $\underline{w \quad m}$) from each of several promising pairs, to perpetuate the promising lines. Hence, in table 1, the F_2 sibs came from the selected F_1 pair, no. 15, and were mated en masse, giving 25.46 per cent crossovers. The F_3 offspring were then mated in pairs, and selection was again exercised. This means that an odd-numbered generation (F_1, F_3, etc.) in table 1, for example, represents the mating of pairs, while an even-numbered generation represents the mating en masse of sibs from the selected pair. It will be clear that inbreeding was very intense throughout all series, for the pair gave sibs, and the sibs from the selected pair mated en masse gave a population in which the most remote relationship could be double cousins, but it might be as close as sibs again. Thus we had alternate generations of double cousins (or nearer relatives) mated in pairs of which we selected the offspring (a sibship) of the most promising pair to mate en masse. Selection therefore really took place in alternate generations. While we recognized that this procedure was not ideal theoretically, at least from the point of view of a strict selection experiment, the advantages outweighed the disadvantages, inasmuch as it made the whole selection experiment possible in a practical sense and yet maintained inbreeding. The chief disadvantage lies in the fact that this method precludes calculation of the parent-offspring correlation and regression coefficient for any two successive generations.

In all these selection experiments, after the P_1 generation, all of the matings were of the type $\frac{w \quad m}{W \quad M} \times \underline{w \quad m}$; i.e., red long females heterozygous in white miniature mated to white miniature males, except where special tests were made for the sake of genetic analysis. This type of mating gave crossovers among the offspring of both sexes and thus a more effective criterion for selection, since numbers were doubled. It also gave the doubly heterozygous females and the ultimate recessive

males as two of the four most frequent classes, which was very convenient, since these were used again for mating in the next generation. Writing the form of all matings for every generation in tables 1 to 6 in the usual Mendelian terms we have:

$$\frac{\text{w} \quad \text{m}}{\text{W} \quad \text{M}} \times \frac{\text{w} \quad \text{m}}{\phantom{\text{W} \quad \text{M}}}$$

red long ♀   white miniature ♂

$$\frac{\text{w} \quad \text{m}}{\text{w} \quad \text{m}} + \frac{\text{w} \quad \text{m}}{\text{W} \quad \text{M}} + \frac{\text{w} \quad \text{m}}{\text{W} \quad \text{m}} + \frac{\text{w} \quad \text{m}}{\text{w} \quad \text{M}}$$

white miniature ♀ + red long ♀ + red miniature ♀ + white long ♀

$$\frac{\text{w} \quad \text{m}}{} + \frac{\text{W} \quad \text{M}}{} + \frac{\text{W} \quad \text{m}}{} + \frac{\text{w} \quad \text{M}}{}$$

white miniature ♂ + red long ♂ + red miniature ♂ + white long ♂

Non-crossovers   Crossovers

THE DATA

Series A; low selection

Table 1 and text figure 1 give the main facts of this selection experiment. The F_1 generation consisted of twenty-eight pairs whose total progeny showed 27.11 per cent crossovers. This is a little lower than might be expected in a general population, but the difference between this ratio and Sturtevant's (given in Morgan and Bridges, '16) ratio of 32.8, based on 41,034 progeny, is no greater than that recorded by Bridges (Morgan and Bridges, '16), who gives data showing 38.3 per cent crossover. This same stock has repeatedly given crossover ratios close to 33 per cent.

The crossover values in this and other similar tables are treated as variables and classified in frequency distributions in which the class interval is 3 per cent. The average of each class is placed at the head of the columns, e.g., 1.5, 4.5, 7.5, etc., which means that the class ranges were 0–3, 3–6, 6–9, and so forth. The crossover values in the F_1 ranged from 10 per cent to 36.8 per cent. There is no doubt but that some of these ratios have little meaning, for they are based upon small totals. We have

TABLE I

Series A: low selection

GENERATION	NUMBER OF PAIRS	THE DISTRIBUTION OF CROSSOVER VALUES IN EACH GENERATION														CROSSOVERS	TOTALS	CROSSOVER VALUES	AVERAGE NUMBER OF OFFSPRING PER PAIR	THE SELECTED PAIR GAVE
		1.5	4.5	7.5	10.5	13.5	16.5	19.5	22.5	25.5	28.5	31.5	34.5	37.5	40.5					
F₁	28				1	1	2	1	6	3	4	4	5	1		427	1,575	27.11	56.3	21: 98=21.43
F₂										×						97	381	25.46		
F₃	27				1	*1*			5	8	4	5	1	2		1,037	3,760	27.58	139.3	34:189=17.99
F₄										×						206	787	26.18		
F₅	56	1		1		3	7	*9*	5	9	11	4	2	2	2	2,188	8,660	25.27	154.6	44:210=20.95
F₆									×							197	920	21.41		
F₇	45	10		*1*		1	6	7	5	7	4	2	2			905	4,234	21.37	94.1	9:104= 8.65
F₈								×								125	612	20.42		
F₉	43	*14*	1	2		3	5	4	7	3	4					478	2,899	16.49	67.4	0:131= 0.00
F₁₀		×														0	53	0.00		
F₁₁	5	*5*														0	87	0.00	17.4	0: 12= 0.00
F₁₂		×														2	148	1.35		

Per cent of crossovers

Generations

Fig. 1   Series A, A′, and B, low selection

recourse to at least two methods of dealing with such unproductive pairs. We can either include in our frequency distribution only those females on which we have ample data to give a somewhat reliable crossover value and ignore all pairs giving less offspring than a fixed minimum (fifty individuals for, example), or we can simply include all females and thus withhold no data. The latter course seemed preferable and we have followed it. There were five pairs showing lower crossover values than the one we selected, as follows: 10.0; 12.5; 16.0; 16.6; 20.7. We did not always select the lowest absolute value, for in many cases this was based upon an insufficient number of offspring. It was also necessary to keep fertility in mind, in order to insure the perpetuation of our selected line. This explanation will make clear why we could not always choose the lowest absolute crossover value in the frequency distribution of any given generation. In table 1 the italicized frequency in each distribution shows the relative position and value of the selected pair. No dispersion can be given for the F_2, F_4, F_6, etc., since these represent en-masse matings. An x represents the point to which the progeny of the pair selected in the preceding generation regressed. The average number of offspring per pair shows how reliable the crossover values usually were in this experiment. The crossovers, total, and the crossover value for each selected pair are also given in the last column. Those generations which have any number of pairs entered under that heading are generations in which all matings consisted of pairs, while the other alternating generations were en-masse matings. Since the crossover value of a female may be based upon a small number of offspring in some cases, and thus give an apparently wide deviation which has little significance, we have not calculated the variability of each generation in this paper. For example, a female showing a crossover value of 10 per cent based upon twenty offspring might well show 30 per cent if one hundred and fifty offspring had been secured, since age and changing temperature affect crossing over; or she might even show 10 per cent based upon twenty offspring as a sheer fluctuation of sampling.

The first two selections seemed to show little or no effect. Although the values of the selected pairs were low, their progeny regressed practically to the parental average. Possibly this means that all wide deviations were not necessarily due to genetic causes and that we had difficulty in distinguishing between wide environmental variates and wide variations due to genetic causes. Selection was thus effective only when by chance we chose a wide variate due to the latter set of causes. For example, in the F_3, we chose a female showing 17.99 per cent crossovers, but her progeny gave an average of 26.18 per cent. After the F_5, progress was very rapid. The F_9 gave 16.49 per cent, and the F_{10} to F_{13} gave about 0 per cent. These last generations in this series were based upon small totals, because the excessive heat (90° to 100°F., day and night) for long-continued periods reduced fertility to a minimum and eventually annihilated our stock in this one. However, series A′, which was derived directly from series A, gave just as low crossover values with larger numbers and under better conditions. We may be quite sure that temperature was not the cause of low crossing over; for, if we may anticipate, series B showed effects of selection under normal temperature conditions.

Series A′, low selection; derived from series A

In the F_7 generation of series A, two selections were made. One female (♀ 14) gave 9:104 = 8.65 per cent, and a second female (♀ 10) gave 1:91 = 1.10 per cent. The former was used to continue series A, while the latter was used to begin a new series, A′. Table 2 and text-figure 1 give the main facts pertaining to series, A′. We began this series to insure keeping alive some of the low crossover material of series A during continuously hot weather. Our facilities did not permit controlling temperature, and the whole experiment was in a precarious situation during the early summer months of 1916. We found that mating a number of females en-masse assured more progeny than the same number of females mated in individual bottles — evidently because the larger number of larvae carried the yeast through the culture and kept molds down. Hence, during the

summer months of 1916, we made numerous en-masse matings in this series to insure keeping the stock alive. Beginning with a single F_7 pair of series A showing 1:91 = 1.10 per cent, the new series A′ was run for nine generations. All generations were en-masse matings except F_9 and F_{14}, in which paired matings were made to ascertain what the crossover values of the individual females might be in this line. In the F_9 the average crossover value for the total population was 8:397 = 2.02 per cent. The wide dispersion in this generation does not carry

TABLE 2
Series A′: derived from series A

GENERA-TION	NUMBER OF PAIRS	THE DISTRIBUTION OF CROSSOVER VALUES IN EACH GENERATION							CROSSOVERS	TOTALS	CROSSOVER VALUES
		1.5	4.5	7.5	10.5	13.5	16.5	19.5			
F_7		1							1	91	1.10
F_8		×							1	86	1.16
F_9	18	*14*	2		1			1	8	397	2.02
F_{10}		×							0	61	0.00
F_{11}		×							0	0.00	133
F_{12}		×							4	373	1.07
F_{13}		×							9	1,473	0.61
F_{14}	25[1]	25							10	2,253	0.44
F_{15}		×							0	289	0.00
Total..									33	5,156	0.64

[1] See text.

much weight because cultural conditions were poor and fertility was low. Pair no. 4, for example, gave 3:15 = 20 per cent, but such a pair might well give a much lower crossover value with a larger number of offspring. The F_{14} gave 10:2253 = 0.44 per cent, and the numbers are large enough to be significant. This generation included twenty-five pairs which gave a total of 2:977 = 0.20 per cent, and an en-masse mating which gave 8:1276 = 0.63 per cent. There can be no doubt but that an original crossover value of 33 per cent has been changed by selection, at least, that a marked change has followed selection. For nine generations the stock bred true to about 0 per cent crossover. The totals for series A′ were 33:5156 = 0.64 per cent.

Series A', like series A, was eventually lost in the latter part of the summer of 1916 because of an unavoidable succession of events. We regretted the loss of this stock because we had hoped to make a genetic analysis of the last generations in an attempt to learn what was taking place during selection. However, the data as they stand indicate that crossing over is not a very stable phenomenon and that it can be rather easily modified. We surely cannot concur in Morgan's ('19) view that crossing over "gives numerical results of extraordinary constancy."

We immediately began a new selection experiment, hoping that we could duplicate the results of series A and A'.

Series B; low selection

Series B, like the preceding series A and A', began with the mating of a single white miniature female and a wild red long male. In fact, as a prelude to series B, we made eighty such paired matings, for we had found some non-disjunction in our original stocks and in series A and A'. Since non-disjunction theoretically lowers the percentage of crossing over (Bridges, '16), we wished to assure ourselves, if possible, that this cause might not be operative in producing low crossover values in our selection experiment. Of the eighty white miniature females tested we found eleven giving either matriclinous daughters or patriclinous sons or both. This must mean secondary non-disjunction in the white miniature stock, for the exceptions were too numerous to be considered primary. We chose white miniature ♀ 53 mated to a wild male as the foundation pair for our experiment, because this pair gave fifty-two wild-type daughters and seventy-eight miniature white sons. While they showed no exceptions, it does not prove that ♀ 53 may not have been non-disjunctional (XXY), for a ratio of 0: (52 + 78) might well occur as a chance ratio where an average of 4.3 per cent of exceptions is expected from XXY females (Bridges, '16). However, in the present paper we are concerned only with the question whether selection based on variable crossover ratios can be effec-

tive. Whether non-disjunction has any necessary relationship to our result will be discussed in a subsequent paper.

TABLE 3

Series B: low selection

GENERATION	NUMBER OF PAIRS	THE DISTRIBUTION OF CROSSOVER VALUES IN EACH GENERATION														THE TOTAL POPULATION IN EACH GENERATION			AVERAGE NUMBER OF OFFSPRING PER PAIR	THE SELECTED PAIR OR SELECTED EN MASSE GAVE
		1.5	4.5	7.5	10.5	13.5	16.5	19.5	22.5	25.5	28.5	31.5	34.5	37.5	40.5	Crossovers	Totals	Crossover values		
F₁	34							3	5	3	10	6	3	2	2	2,056	7,189	28.60	211.4	61: 219=28.75
F₂										1						129	530	24.34		129: 530=24.34
F₃	47			1	1		1	9	5	8	7	6	6	2	1	2,204	8,089	25.02	172.1	175: 645=27.13
F₄											1					330	1,141	28.92		330:1141=28.92
F₅	82	1	1	1			4	19	17	5	19	11	2			5,798	23,618	24.55	288.0	48: 230=20.87
F₆									1	1						379	1,411	26.86		296:1130=26.19
F₇	61	1			1	1	4	4	18	13	9	8	1	1		5,490	21,974	24.98	360.0	36: 251=14.34
F₈									1	1	1	1				650	2,858	22.74		217:1185=18.31
F₉	83			1	12	12	13	17	13	9	3	2			1	3,230	17,550	18.40	211.4	17: 276= 6.16
F₁₀					1	2	2	1								595	3,502	16.99		191:1179=16.20
F₁₁	84		3	6	13	24	14	10	10	3	1					1,896	11,937	15.88	142.1	5: 96= 5.20
F₁₂						3	3	1	1							815	4,558	17.88		66: 512=12.89
F₁₃	63	3		9	13	16	10	6	5							759	7,439	10.20	118.1	15: 197= 7.61
F₁₄					1	3		1					1			149	790	18.86		25: 207=12.08
F₁₅						1										68	609	11.17		68: 609=11.17
F₁₆	79	5		7	22	29	12	2	1	1						1,416	14,765	9.59	186.9	12: 169= 7.10
F₁₇					6	1										642	6,027	10.65		122:1009=12.09
F₁₈	74	6		5	28	22	9	1	2	1						1,195	12,166	9.82	164.4	4: 77= 5.19
F₁₉					3	9	4	2								975	8,786	11.10		47: 464=10.13
F₂₀	52	4		5	12	16	9	5	1							641	6,533	9.81	125.6	20: 179=11.17
F₂₁				1	5	5										1,108	11,407	9.71		199:2027= 9.82
F₂₂	67	3		6	15	22	12	7	2							1,232	11,717	10.51	174.9	1: 63= 1.59
F₂₃				1	2	2		1								392	3,649	10.74		51: 768= 6.64
F₂₄	47	10	13	16	5	3										431	6,801	6.34	144.7	0: 107= 0.00
F₂₅			1	1	4	1										223	3,119	7.15		39: 454= 8.59
F₂₆	38	5	13	12	6	1		1								280	3,903	7.17	102.7	6: 141= 4.26
F₂₇			1	1												65	1,152	5.64		43: 873= 4.93
F₂₈	46	10	8	12	7	6	3									215	2,650	8.11	57.6	0: 39= 0.00
F₂₉				2												10	158	6.33		8: 129= 6.20

The F₁ sibs from ♀ 53 were mated in thirty-four pairs and gave as a whole a crossover value of 28.60 per cent (table 3).

In order to further test our foundation stock, the F_1 offspring of ♀ 25 (one of the eighty P_1 ♀ ♀, and similar to ♀ 53) were tested en-masse and gave $1142 : 3553 = 32.14$ per cent. The F_1 offspring of several other F_1 ♀ ♀ were mated en-masse and gave $830 : 2923 = 28.40$ per cent. All of these facts indicate that our foundation stock was quite normal with respect to crossing over and gave crossover values of the same general magnitude as those ordinarily used in plotting maps of the sex chromosome.

The main facts pertaining to series B are given in text figures 1 and 2 and in tables 3 and 4. Table 3 was constructed in the

Per cent of crossovers

Generations

Fig. 2   Series B, continued, low selection

same way as table 1, with the following exception: in series B, records of the en-masse matings of the offspring from several promising pairs were kept and the crossover values of all these are put in the form of a frequency distribution, but the italicized frequency shows the position of the en-masse mating which was derived from the pair eventually selected to continue the experiment. The italicized frequency in the distribution of the pairs likewise shows what the value of the chosen pair was. The first three columns at the right of table 3 give the data for the total population in each generation. The average number of offspring per pair shows that the fertility was high and selection was based upon what seemed to be adequate numbers. The last column gives the number of crossovers total, and crossover value

of the selected pair in each generation and the same data for en-masse matings from these selected pairs. Text figures 1 and 2 give a graphic representation of the progress made in series B. The graphs are based upon the crossover values in the selected line; i.e., all en-masse matings except the selected one have been neglected in plotting the graph. In other words, the graph relates only to the actual line of selection, and all side lines have no weight in determining the coordinates. It will be clear that those generations in table 3 which have any number of pairs entered under that heading were generations made up entirely of paired matings, while all other generations were en-masse matings.

The first three selections had little or no effect, but it cannot be said that selection was very rigid during these generations. In F_7 we selected a pair giving $36:251 = 14.34$ per cent and made some progress, for the next seven generations (F_8–F_{14}) fluctuated between 10 per cent and 23 per cent. The subsequent nine generations (F_{15}–F_{23}) fluctuated around 10 per cent. Selection was carried on up to F_{29} and the last six generations (F_{24}–F_{29}) varied around 6 per cent.[4] After that we simply carried the stock without selection, and have found it to breed quite true to low crossover for twenty-two generations. The F_{29}–F_{50} have given values around 6 per cent. These last twenty-one generations are shown in table 4.

There are some features of tables 3 and 4 which require comment for the sake of clearness. Temperature conditions made it necessary to breed the offspring of the selected pair in the F_{13} for two generations by the use of en-masse matings. Hence, the matings in the F_{14} and F_{15} show no pairs and selection was interrupted. This was the only case in which the usual sequence of selecting in alternate generations was not followed. The F_{33} showed a rather abrupt rise in crossover value (12.50 per cent),

[4] An independent mutation of gray to yellow which occurred in the F_{25} should perhaps be put on record. One female ($♀$ no. 30) proved to be heterozygous for yellow, and this gene was linked to white and miniature. Hence the mutating gene came through the spermatozoon from the gray white miniature father of $♀$ 30. This new gene for yellow proved to be identical with the original yellow mutation found by Wallace in 1911 (Morgan and Bridges, '16).

which was without doubt due to high temperature, as our records indicate. The fertility was low and we obtained with much effort from en-masse matings in the F_{32} and F_{33} only forty-eight and eighty individuals, respectively, while under ordinary conditions several thousand would have been possible. As soon as normal conditions were restored, the usual low crossover values were again found. The F_{41} showed a rather unexpected rise

TABLE 4

Series B—Continued

GENERATION	CROSSOVERS	TOTALS	CROSSOVER VALUES
F_{30}	6	144	4.17
F_{31}	7	171	4.09
F_{32}	4	48	8 33
F_{33}	10	80	12.50
F_{34}	52	643	8.09
F_{35}	48	1,147	4.18
F_{36}	55	1,032	5.33
F_{37}	46	814	5.65
F_{38}	39	697	5.60
F_{39}	55	954	5 77
F_{40}	72	1,074	6.70
F_{41}	94	1,015	9 26
F_{42}	463	8,564	5.41
F_{43}	47	901	5.22
F_{44}	103	1,312	7 85
F_{45}	43	661	6 51
F_{46}	59	992	5 95
F_{47}	69	1,021	6.76
F_{48}	45	734	6.13
F_{49}	81	1,081	7 49
F_{50}	96	1,375	6.98

(9.26 per cent), but since there were no unusual temperature conditions, we must regard this somewhat higher value as without peculiar significance. The subsequent generations dropped to about 6 per cent again.

Series C; high selection

In the F_1 generation of series A, pair number 21, was chosen to begin a high-selection series, series C. While this series was carried for only eight generations, and then discarded in order to devote time to the other series, nevertheless brief mention should be made because the results may aid us in interpreting series A, A′, and B. We were not able to make progress in selecting upward, as the averages of table 5 show. (Table 5 was constructed in the same way as tables 1, 2, and 3.) On the contrary, we were much surprised to find that in the F_7 generation a number of pairs suddenly dropped to very low

TABLE 5
Series C: high selection

GENERATION	NUMBER OF PAIRS	CROSSOVERS	TOTALS	CROSSOVER VALUES
F_1	28	427	1,575	27.11
F_2		162	512	.31.64
F_3	35	1,407	4,842	29.06
F_4		436	1,355	32.18
F_5	90	6,465	21,071	30.68
F_6		684	2,267	30.17
F_7	72	3,893	13,705	28.41
F_8		296	1,298	22.80

crossover values; in fact, much lower ratios than one would find in any ordinary population such as our original stocks or our F_1 of table 1. The distribution of the F_7 in series C is given in table 6. It will be noted that nine pairs gave values lower than 6 per cent. Their values were as follows:

$$
\begin{aligned}
4{:}72 &= 5.56 \\
5{:}279 &= 1.79 \\
9{:}164 &= 5.49 \\
1{:}142 &= 0.70 \\
1{:}82 &= 1.22 \\
0{:}123 &= 0.00 \\
4{:}104 &= 3.85 \\
2{:}40 &= 5.00 \\
0{:}49 &= 0.00 \\
\hline
\text{Total, } 26{:}1055 &= 2.46
\end{aligned}
$$

There can be no doubt that these crossover values are significantly different from any ratio in the F_1 in table 1, or from the usual ratios shown by random stock females. Furthermore, there is an interval of about 10 per cent between the lower and higher groups of table 6, in which we found no crossover values. The natural inference is that any attempt to increase the amount of crossing over leads to double crossing over, and thus to very low crossover values (practically zero). That is, these nine females showed a marked decrease in crossover values, despite high selection, because they gave almost nothing but double crossovers. In other words, their low crossover values are, after all, the result of effective high selection. Mr. L. E. Thorne, who had this series under observation, was called into military service and we did not make any further tests on this material.

TABLE 6

The distribution of crossover values in the F_7 generation of series C

NUMBER OF PAIRS	THE DISTRIBUTION OF CROSSOVER VALUES															CROSSOVERS	TOTAL	CROSSOVER VALUE
	1.5	4.5	7.5	10.5	13.5	16.5	19.5	22.5	25.5	28.5	31.5	34.5	37.5	40.5	43.5			
72	5	4				1	1	6	8	15	16	8	6	1	1	3,893	13,705	28.41

We hope, however, to repeat the high-selection experiment and test out the region between white and miniature in such females which apparently give uniform double crossing over in a region in which single crossing over is the rule.

DISCUSSION AND SUMMARY

As far as we are aware, there is only one record of a similar selection experiment. Gowen ('19) selected for high and low crossover values, but his results and conclusions are diametrically opposed to ours, since he found selection ineffective, and concluded there were no differences in modifying factors for crossing over in his experiment. He continued selection for only five generations in the low series and six in the high, using the region of the third chromosome between sepia and rough.

While it is possible that this chromosomal region may fail to show the same phenomenon which we found in the sex chromosome, we are rather inclined to believe that the difference between our results and Gowen's is more likely due to differences in the method of procedure, for Gowen states that his "chief difficulty lies in the few individuals that it was possible to include in a given generation." Gowen gives only the mean total crossing over in each generation, and we do not know how rigid his selection may have been, for he does not state how many pairs were included in each generation nor does he give the frequency distribution for crossover values. We suspect that he found the same impediments in using strict brother-and-sister matings which we found and which prompted us to use en-masse matings in alternate generations to increase our numbers. We are carrying on selection experiments in other regions of the sex chromosome and in the autosomes, which should decide whether other regions and chromosomes are similarly affected by selection. We have no reason to suppose that they will not be.

The effects of selection upon crossover values may be due to one or a number of causes, some of which suggest themselves almost immediately. It would hardly be profitable to expatiate on these, since we are making tests, which we hope may indicate what has really happened in the course of selection. Briefly stated, we think of the following possible causes which may have been operative in modifying our crossover values:

1. We may perhaps have dropped out a large part of the chromosome between white and miniature, thus bringing these two genes closer together. We can probably disregard this as a cause, for although 'deficiency' reduces crossing over (Bridges, '17) nevertheless the lethal action of deficiency would be seen in a disturbed sex ratio. We found no such disturbance.

2. Is it possible that we may have moved the locus of the genes on the chromosome? This would mean that the locus of a gene is not permanently fixed, but that a given gene is found in a characteristic position in the majority of cases. If we have done this, and at the same time have not moved other genes, then linkage tests should disclose this fact, for the order of the genes would be changed.

3. In series A and A' we found much evidence of non-disjunction. Bridges ('16) stated that XXY females should logically show a decrease in crossing over, because heterosynapsis takes place in about 16.5 per cent of the cases and precludes crossing over in these cases. However, Bridges also showed that the experimental results disagree with such an expectation, for crossing over was not decreased among the regular sons of XXY females, but as far as the evidence goes it was slightly increased. For some time we labored under the impression that much, if not all, of our decreased crossing over was associated with the presence of non-disjunction (Detlefsen and Roberts, '20). We are now rather inclined to believe we were in error. It should not be a difficult matter to free our low crossover stock in series B from non-disjunction and thus dissociate this possible cause from the others. We could in this way demonstrate that non-disjunction was only accidentally present in our experimental material and that our results are quite independent of non-disjunction.

4. Have we reduced the frequency of the usual single 'chromosome twist' between white and miniature to a minimum? Weinstein's ('18) results indicate that crossing over takes place in the sex chromosome with about forty-six units as the modal distance between successive crossovers. Similarly, Gowen ('19) found twenty-five to thirty units in the case of the third chromosome. We began with two genes which were about thirty-three units apart, and which should therefore show a single crossover as the characteristic or modal number. This would mean that in series A, A', and B we have practically eliminated the usual single crossover in this region, while in series C we were on the way to increasing it to two crossovers (i.e., a double crossover), which would give us no crossing over as far as these two genes were concerned. Does this mean that we can decrease or increase the amount of 'twisting' which members of an homologous pair of chromosomes may show, and which is supposed to be responsible for crossing over according to the chiasmatype theory? If selection can accomplish this, then we may reasonably suppose that numerous hereditary modifying factors are

present in a general population and are the basis and cause of
this variable chiasmatype relationship. If this explanation is
correct (and we are inclined to believe it the most plausible one
of those we have suggested here), then we cannot escape a
marked change in our view-point on crossing over and related
phenomena. If, for example, all of the difference between prac-
tically no crossing over in our series A and A′ and normal cross-
ing over (33 per cent) is due to numerous modifying factors, then
we naturally begin to wonder just what part 'distance between
two genes' on a chromosome may play in determining linkage
values. Our current view is that "the percentage of cases in
which two linked genes separate (amount of crossing over between
them) is necessarily proportional, other things being equal, to
the distance between the genes," (quoted directly from Weinstein
(′18)). But evidently the percentage of crossing over is a vari-
able which is the expression of different possible combinations
of multiple modifying factors; hence the percentage of crossing
over cannot be proportional to the distance if the distance
remains uniform. For example, in series B we find 6 per cent
crossing over, and so we should conclude that the distance in
this stock is 2/11 or 18 per cent of what it was when we began
selection! Thus, to maintain our original position, we must
conclude that the percentage of crossing over and distance are
correlated variables, if the proportion between the two is to
remain reasonably constant. We then naturally begin to wonder
what has happened to all of the distance (and the genes) between
0 and 33 in series A and A′ where crossing over has been prac-
tically eliminated. In view of these considerations, it would
perhaps be simpler to conclude that the percentage of crossing
over is not necessarily proportional to the distance. The dis-
tance may remain fairly constant, but the amount of crossing
over (i.e., twisting of the chromosomes) will depend upon numer-
ous hereditary factors.

One recalls in this connection Goldschmidt's (′17) suggestive
paper in which he postulated variable forces that hold genes to
their customary loci on the chromosome and which allow an
exchange between allelomorphs in a certain average percentage

of cases. While we cannot subscribe fully to this theory for cogent reasons advanced by Sturtevant ('17), Bridges ('17), and Jennings ('18), nevertheless Goldschmidt's proposed theory would not appear entirely supererogatory, for a crossover value is apparently a variable and the variation is due to or controlled by multiple hereditary factors. A cross between low crossover stocks and the original population, and testing out a large number of F_2 segregates should throw the desired light on this question. Unpublished data indicate that segregation in crossover values does take place as one would expect on the basis of the multiple-factor explanation.

5. May we suppose that we have been taking advantage of small mutations in the nature of modifying factors arising during the course of selection? While this is possible we are inclined to doubt it, for favorable mutations evidently do not take place in the direction of selection as readily as this view would imply (cf. Muller and Altenburg, '19).

The following conclusions may be drawn from the data of this paper:

1. Crossover values are very variable and part of this variability is due to genetic causes.

2. Low selection has been effective in two entirely independent series, A and B.

3. The low crossover stock bred true to about 0.6 per cent (almost zero) for nine generations in series A' (derived from series A).

4. The low crossover stock bred true to about 6 per cent for twenty-two generations in series B.

5. High selection probably induces double crossing over, as shown by series C.

6. Crossing over in the various regions of the sex chromosome (and other chromosomes?) is probably controlled by multiple incompletely dominant factors.

LITERATURE CITED

BRIDGES, C. B. 1915 A linkage variation in Drosophila. Jour. Exp. Zoöl., vol. 19, pp. 1–21.
1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics, vol. 1, pp. 1–52, 107–163.
1917 An intrinsic difficulty for the variable force hypothesis of crossing over. Am. Nat., vol. 51, pp. 370–373.
1917 Deficiency. Genetics, vol. 2, pp. 445–465.
DETLEFSEN, J. A. AND ROBERTS, E. 1920 Variation in the percentage of crossovers and selection in Drosophila melanogaster. Anat. Rec., vol. 17, p. 336.
GOLDSCHMIDT, R. 1917 Crossing over ohne Chiasmatypie? Genetics, vol. 2, pp. 82–95.
GOWEN, J. W. 1919 A biometrical study of crossing over. On the mechanism of crossing over in the third chromosome of Drosophila melanogaster. Genetics, vol. 4, pp. 205–250.
JENNINGS, H. S. 1918 Disproof of a certain type of theories of crossing over between chromosomes. Am. Nat., vol. 52, pp. 247–261.
MORGAN, T. H., AND BRIDGES, C. B. 1916 Sex-linked inheritance in Drosophila. Publ. Carnegie Inst. Wash. D. C., no. 237, pp. 1–87, 8 fig., 2 pl.
MORGAN, T. H. 1919 The physical basis of heredity. J. B. Lippincott Co., Philadelphia and London. 305 pp., 117 fig.
MULLER, H. J., AND ALTENBURG, E. 1919 The rate of change of hereditary factors in Drosophila. Proc. Soc. Exp. Biol. and Med., vol. 17, pp. 10–14.
PLOUGH, H. H. 1917 The effect of temperature on crossing over in Drosophila. Journ. Exp. Zool., vol. 24, pp. 147-210.
STURTEVANT, A. H. 1917 Crossing over without chiasmatype. Genetics, vol. 3, pp. 301–304.
1919 Inherited linkage variations in the second chromosome. Pub. Carnegie Inst. Wash. D. C., no. 278, pp. 305–341.
WEINSTEIN, A. 1918 Coincidence of crossing over in Drosophila melanogaster (ampelophila). Genetics, vol. 3, pp. 135–172.

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Experimentos sobre el efecto de la extirpación del pronefros de
Amblystoma punctatum.

Las condiciones que siguen a la extirpación bilateral del pro-
nefros de Amblystoma punctatum demuestran claramente que
estos órganos son necesarios para la vida del embrión. Todos
los embriones desprovistos de riñones cefálicos presentan debili-
dad cardíaca y edema, muriendo al cabo de doce días. La
doble extirpación de los túbulos pronéfricos no afecta al desa-
rrollo normal de los glomérulos. La presencia de un solo pro-
nefros es suficiente para mantener la vida del animal. Después
de las operaciones unilaterales el resto del pronefros presenta
una marcada hipertrofia, aumentando un 100 por ciento el
área de la superficie secretora; el contenido cúbico de la masa
de células 63 por ciento, y la longitud de los túbulos 21 por ciento
sobre lo normal.

El conducto segmentario procedente del órgano hipertrofiado
posee un dámetro medio mucho mayor que el de cualquiera de
los dos conductos del animal normal. El riñón hipertrofiado
también presenta indicios de una pequeña cantidad de hiper-
plasia, puesto que el número de núcleos presente en él es 16 por
ciento mayor que el normal. En el lado operado los glomérulos
se desarrollan normalmente, y los embudos anterior y posterior
pueden regenerar a expensas del epitelio celómico. La condición
del conducto segmentario varía considerablemente. En los
casos extremos está representado solamente por una pequeña
masa de células degeneradas. En los embriones en que se ha
extirpado el rudimento del corazón en un estado muy temprano
del desarrollo, el desarrollo inicial de los glomérulos es normal,
pero pronto se altera a causa de la enorme distensión de los
vasos sanguíneos.

EXPERIMENTS ON THE EFFECT OF REMOVAL OF THE PRONEPHROS OF AMBLYSTOMA PUNCTATUM[1]

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TWENTY-THREE FIGURES

CONTENTS

INTRODUCTION

The common occurrence, among the lower orders of vertebrates, of a more or less persistent head kidney or pronephros has led to the accumulation of a very comprehensive literature on this subject from the standpoint of pure morphology. Little evidence has been furnished in any group, however, of the rôle which these organs play in the life of the embryo.

Price ('10), working on the head kidney of one of the myxinoids, Bdellostoma stouti, a form in which it persists throughout adult life, follows up his earlier descriptions of the development

[1] A preliminary report of the results obtained was published in 1916. (Howland, R. B., '16. On the Effect of Removal of the Pronephros of the Amphibian Embryo. Proc. Nat. Acad. Sc., vol. 2.)

of the excretory system with a study of both the structure and the function of this organ in its fully formed state. The head kidney of Bdellostoma is shown to be a composite structure, possessing at its earliest appearance all the characteristics of a pronephros, with the single exception of the typical glomerulus, but later fusing with the anterior end of the mesonephros and losing all connection with the exterior. Structurally, then, the head kidney in at least one of the myxinoids is rendered incapable of playing the rôle of an excretory organ, but since it is connected with both coelomic cavity and the circulatory system, and since, also, it has been proved possible to transfer certain substances from the coelomic fluid directly into the circulation, Price concludes that its probable function is the transference of lymph from the body cavity into the blood-vessels.

Since the discovery of the organ by Johannes Müller ('29), the origin, development and morphology of the amphibian pronephros have been described by many investigators, chief among whom are von Wittich ('52), Fürbringer ('78 a), and Field ('91). An excellent review of the early controversies concerning its structure is also given by Field. The presence of a well-developed pronephros in the amphibian embryo, its early appearance, and its relatively large size have led to the general assumption that it is a functioning organ. Its characteristic structure, consisting in a glomerulus which extends freely into the coelomic cavity, a coiled tubule furnished with open ciliated funnels for the intake of coelomic fluid, and a simple duct establishing direct connection with the exterior, further points to its function as excretory in nature. Still, from the physiological viewpoint, no experimental evidence as to the extent to which the embryo is dependent upon it for the elimination of excretory products had been offered at the time of publication of my first note ('16); Since then Swingle (19), working independently upon the embryo of Rana sylvatica, has obtained results which in the main agree with my own.

In the series of experiments described in the present paper, the necessity of these organs for the life of the embryo has been proved by the fact that death follows, in time, after the removal

of both pronephroi. The uniform occurrence of a pronounced edema after bilateral extirpation, similar to the condition which follows certain pathologic conditions in the permanent kidneys of the higher animals, suggests a further parallel between the larval kidneys on the one hand and the permanent kidneys on the other with respect to their function. Extirpation of the coiled portion of one or both pronephroi has also afforded the opportunity of investigating the question of correlation in development through a study of the effect of its removal on the other components of the excretory system. The response of one kidney in cases where it has been left functioning alone has further led to the consideration of the factors involved in the restoration of the normal secreting area through the process of compensatory hypertrophy.

Although no invariable rule can be formulated as to the type of regulation which may be anticipated as a consequence of the abnormal conditions imposed by extirpation of an embryonic region or organ, a survey of the results obtained in the many instances already investigated shows that, in a large proportion of cases, there occurs a more or less complete regeneration of the excised part. Byrnes ('98 b) and Harrison ('18) have shown, for instance, that the limbs of the amphibian embryo, if removed at an early age, will soon be replaced through the regenerative capacity of the surrounding tissue. This is also true of the auditory and nasal placodes, the lens, and the gills. The possession by the amphibia, of the regenerative power to such a high degree would naturally lead to the presumption that removal of the pronephric rudiment might result in a similar replacement of this organ. This, however, as will be shown later, is not the case in the Amblystoma larva, for the adjustment consequent on removal of the pronephros is not in the nature of a restitution, but is a compensating hypertrophy[2] of the remaining head kid-

[2] A peculiar instance of compensatory hypertrophy in another organ is cited by Kochs ('97) in his work on Triton, where the amputation of the fore leg often resulted in a marked hypertrophy of the tail. Retardation or acceleration in the growth of a fore leg in the larvae of Rana esculenta and Bufo viridis may be induced by removal of a hind leg, according to Kammerer ('05), the hastening or arrest of growth depending on the rapidity of wound healing.

ney and of its duct—such compensation as is common in the adult kidney of the higher vertebrates. The degree of compensation which has been attained by the single kidney has been estimated in terms of increase in the secreting surface of the pronephric tubules, as well as by measurement of the volume of the cells making up the walls.

Although the literature on the subject of hypertrophy in the kidney of the higher forms is too extensive to permit of a discussion in any detail in the present paper, it may be well to mention several of the more important standpoints from which the subject has been treated.

The question of direct causal connection between the demand for increased functional activity and the changes in the compensating organ has been definitely settled by such experimental studies as those of Sacerdotti ('96), in which the kidneys of unoperated dogs were stimulated to compensatory overgrowth by injection of the blood from completely nephrectomized animals. In this instance, as in all the early pathological literature dealing with this subject, the exact nature of the histological changes evoked in the stimulated organ was given only secondary consideration. Recently, however, with the general acceptance of the distinction between the terms hypertrophy and hyperplasia, more accurate observations of the condition of the kidney constituents consequent on increased activity have been made. Both forms of regulation may occur in the same organ, each being limited to a definite area. Wolff ('00), in his contributions on the macroscopic and microscopic conditions of the hypertrophied kidney after resection, draws a sharp distinction between those changes which occur in the region of the lesion, and those occurring in the uninjured portions of a resectioned kidney. In the former location he observes that mitoses appear at the end of two days, resulting in the formation of new epithelium. In the uninjured portion of the remaining kidney tissue, however, no new formation of either urinary tubules or of glomeruli takes place as compensation for those excised, but here a sufficient restitution occurs through increase in size, and the normal balance is restored. From the histological viewpoint,

this process is sure to be almost entirely one of hypertrophy, not a hyperplasia, of the kidney elements. In the glomerulus and urinary tubules the former process occurs exclusively, in the epithelial cells themselves, hypertrophy with a negligible amount of hyperplasia.

The clearest and by far the most accurate statement of the exact histological conditions found in the hypertrophied kidney is given, however, by Galeotti and Villa Santa ('02). These authors approached the problem from a widely different view-point, their main object being to determine whether the hyper-trophied kidney of an adult animal would show the same histo-genetic potency as that of an animal which had not attained its full growth. From their study of the kidneys of young and adult dogs and rabbits, careful estimates were made of the num-ber of glomeruli found in sections of normal and hypertrophied kidneys and of the relationship between the average surface area of the glomeruli and the number present. Furthermore, the diameter of the lumen in the tubuli recti was accurately measured and the secreting surfaces in the two kidneys obtained for comparison. The volumes of the cell walls in the two cases were also computed and contrasted. The thoroughness of the methods used in these computations gives added weight to their conclusion that, whereas in the young kidney hyperplasia may occur, the adult kidney has lost its potency for addition of new parts, and can only respond by the enlargement of those elements already present.

Kittleson ('20), in his recent report on the effects of inanition and refeeding on the growth of the kidneys in young rats, con-firms the opinion of former workers that starvation inhibits the formation of renal corpuscles, and further concludes that "refeed-ing after stunting results not only in a hypertrophy of the renal corpuscles but also in an increase in number, which may even exceed the normal." This would indicate the possibility of both hypertrophy and hyperplasia of the same kidney element, induced by these abnormal conditions.

No instances are on record of experiments dealing with the production of hypertrophy or hyperplasia in the adult am-

phibian kidney, although Levi ('05) claims that the anuran
mesonephros has, in one species, the power of regeneration after
injury. He destroyed both the urogenital anlage and that
of the wolffian duct and tubules in Bufo larvae by means of a
red-hot needle, and obtained after a certain time complete
regeneration of the excretory organs. His method is, however,
open to the criticism that no accurate estimate of the actual
extent of the injury done to the organs by this type of operation
can be made. The introduction of a hot needle may cause only
minor displacement or destruction of a few cells of the duct or
coils. Certainty as to the degree of injury can be assured
only by complete excision.

Removal of a portion of a given organ or system has been
found to have a varied effect on the formation of its other constit-
uents. The extent to which the growth of one part is influenced
during its development by other developing portions of the
embryo varies widely, the scale of difference ranging from com-
plete interdependence to those extremes in which each con-
stituent possesses the potency for self-differentiation without
the influence of any formative stimuli.

In view of this variation in the degree of correlation exhibited
by closely related parts during their early growth, it was of interest
to consider, particularly in cases of bilateral extirpation of the
pronephros, the effect of the absence of the tubules on the forma-
tion of the glomeruli. The operated animals showed without
exception a differentiation of glomerular tissue perfectly normal
in position and size. The glomerulus therefore possesses the
power of self-differentiation, and is entirely independent of the
presence of the tubular elements.

MATERIAL, METHODS AND NORMAL DEVELOPMENT

Embryos of Amblystoma punctatum were used for all the experiments. The stages chosen for operation varied from the condition in which the first loop of the pronephric tubules appears as a slight, ventrally directed curve of the duct (fig. 1, stage 30)[3] to that in which the two funnels, together with the first loop, appear as a broadened Y (fig. 2, stage 32). In all cases embryos were used before contraction of the body muscles began, as movement not only hindered the operation, but often tore open the wound after successful removal of a kidney.
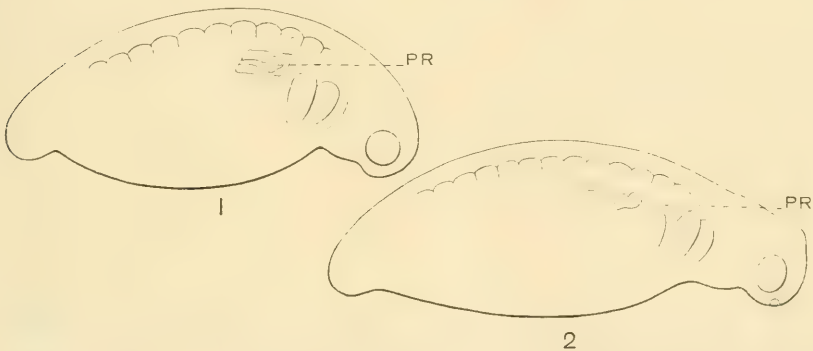
Figs. 1 and 2   Embryos in the stages used for operating. *PR*, pronephros located below the third and fourth myotomes. Figure 1, earliest stage (stage 30); figure 2, latest stage (stage 32).

Anaesthetics were unnecessary, and the slight motion due to the ciliated epithelium was controlled by holding the animal in the field with an operating needle. The body tissues in these early stages are easily distinguished from each other through slight differences in pigmentation, and, in addition, are so loosely bound together as to allow removal of the pronephric mesoderm without dislocating the cells of contiguous regions. In a few instances portions of the somatopleural layer ventral to the pronephric rudiment were included in the tissue removed, resulting in retarded development, in abnormalities, or even in total absence of the limb on this side (Harrison, '18).

[3] See Harrison, R. G., '18, Jour. Exp. Zoöl., vol. 25, no. 2, p. 417, footnote 9.

The general methods employed in operating are so well known that no detailed description of them is necessary here. The special technique required in removal of the pronephros and in the construction and measurement of the models will be described in later sections.

The pronephric swelling is one of the earliest and most clearly defined of the developing organs. Its position may be accurately located at a stage not long after the closing over of the neural folds, when the first eight pairs of muscle plates may be seen, and, like these, it differentiates in an anteroposterior direction. It is found in the region immediately underlying the
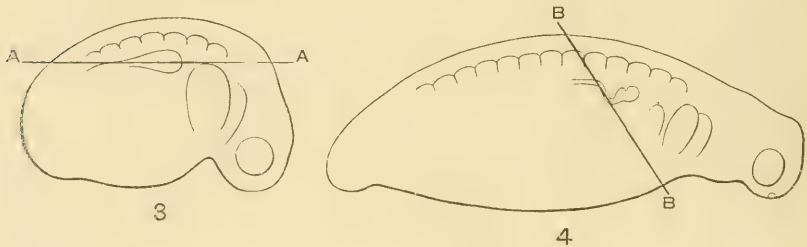
Figs. 3 and 4 Embryos showing the first looping of the tubule, due to rapid growth of the cells just posterior to the nephrostomes. Figure 3, a–a, original axis of the pronephric rudiment. Figure 4, b–b, direction of first bend of the tubule.

third and fourth myotomes as a bulbous thickening tapering posteriorly into a short thickened ridge. Operations at this period, although having the advantage of not interfering with developing nerves or blood-vessels, are inadvisable, since the mesoderm is still so compact that excision results almost invariably in the removal of more than the pronephric rudiment. In succeeding stages, the delimitation of the segmental duct progresses, and the original bulbous enlargement becomes pitted in in two places on its coelomic border, establishing the nephrostomal openings into the anterior and posterior funnels. These two openings lie opposite the midline of somites three and four. At the same time the tubule, which originally lies along the longitudinal axis of the body just below the muscle plates (fig.

3, a—a), lengthens rapidly in the region of the fourth myotome, and bending outward and downward in an acute angle over the upper surface of the yolk (fig. 4, b—b), forms a U-shaped loop. A little later this is bent over anteriorly, and may even come to lie slightly farther forward than the anterior nephrostome.

The pronephros of Amblystoma differs from that of certain of the Anura in the possession of two instead of three nephrostomal canals,[4] and in the absence of the common chamber or 'pronephric pouch,' the funnels, instead, narrowing directly
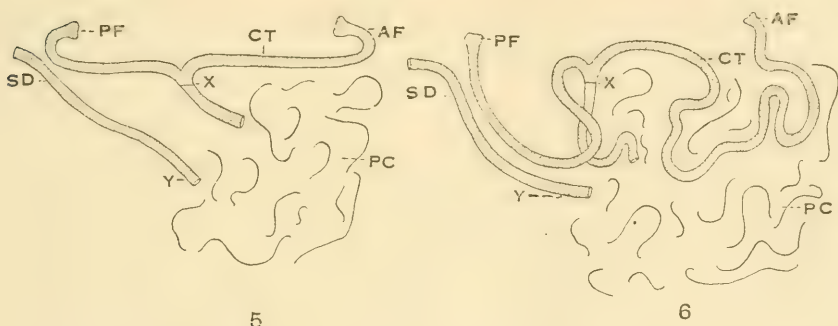
Figs. 5 and 6   Diagrams showing the region where greatest growth occurs in the early and late stages of development of the pronephros. Figure 5, condition before the coiling of the longitudinal tubules connecting the funnels. Figure 6, growth of these tubules in the older kidney. $a.f.$, anterior funnel; $p.f.$, posterior funnel; $c.t$, longitudinal tubule; $s.d.$ segmental duct; x-y, region of greatest growth during early development resulting in the formation of the ventrolateral portion of the pronephric coil, $p.c.$ Shaded areas drawn from wax models, pronephric coil indicated by curved lines.

into the U-shaped tube just described.   With further multiplication of the cells just below the funnels, two longitudinal tubules are established (figs. 5 and 6, $c.t.$, and fig. 17, $L.T.$), separating the anterior and posterior nephrostomes from each other and from their original point of junction with the looped tubule (x), as they grow.   This growth is at first a very slow process as compared with that of the U-shaped portion.   In the latter region the active proliferation of cells results in a rapid

[4] One instance is on record of the presence of a third funnel on both sides in an Amblystoma larva.   See Field ('91).

coiling of the tubule, the early increase in size of the kidney being limited mainly to this region, between the connecting tubule and the proximal end of the segmental duct (figs. 5 and 6, x to y). This eventually forms the ventrolateral region of the fully formed organ, which will again be referred to in connection with the discussion of edema.

In still later stages[5] the nephrostomal canals and their connecting tubule also elongate, and are thrown into loops and folds (fig. 6, $l.t.$), retaining their dorsal position and extending slightly laterally over the coils already formed. This portion may then be termed the dorsolateral region, as contrasted with the ventrolateral portion already mentioned. That part of the tubule which is a direct continuation of the segmental duct never becomes strongly convoluted, but retains its original position along the ventrolateral boundary, slanting obliquely toward the dorsal surface over the kidney from the anterior margin. Minor folds may occasionally occur along its course. Increase in growth is outwardly evidenced by a more and more pronounced swelling in the pronephric region. Operated specimens may be easily distinguished from normal animals, even after healing is complete, through the absence of this thickening on the operated side. Posterior to the pronephric coils, the segmental duct extends backward along the body just below the ventral surfaces of the muscle plates. The junction between pronephric coils and the proximal end of the segmental duct is always in the immediate region of the posterior funnel. With the subsequent downgrowth of the myotomes the formation of the shoulder-girdle and anterior limb buds, the pronephros becomes partly covered, and comes to lie deeper in the body, and nearer the midline of the embryo. The edges of the myotome also extend downward over the segmental duct, making its removal in this stage extremely difficult.

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[5] Excised kidneys in the older stages may be slightly stained, and the capsular nuclei, thus made visible, removed. The tubules may then be easily uncoiled for observation in water or weak alcohol. Oil is an unsatisfactory medium, both for examination and preservation, as it not only increases the brittleness of the tubules, but renders them too transparent for clear definition.

Parallel with this development, although not appearing at so early a stage, the rudiment of the glomerulus is formed. This first appears as a thickening in the opposite or splanchnic wall of the coelom, extending over an area as long as the distance between the anterior and posterior funnels. Vascular cavities soon appear, and at an early stage these become continuous with branches of the dorsal aorta. The arterial supply is derived directly from this source; the venous supply from the postcardinal vein, which enlarges in the region of the pronephros, forming a large venous sinus, into which the fully developed kidney projects. The tubules are thus continually bathed in the blood returning from the posterior part of the body (Field, '91).

BILATERAL EXCISION OF PRONEPHRIC RUDIMENTS

Mode of operation

The first experiments consisted in the removal of the pronephric rudiments on both sides, to test the functional necessity of these primary organs in the life of the embryo.

In the largest proportion of cases, a period of several hours, or even a day, was allowed to elapse between the removal of the right and left pronephros. However, the two excisions may follow each other immediately without incurring serious results.

Sharp-pointed needles, inserted in glass rods, were substituted for the more generally used iridectomy scissors. Controls were kept under identical conditions of light, temperature, water, etc. Two methods were employed in removing the pronephros. In the first, three straight cuts were made, one beneath and one along each side of the pronephric swelling. The flap of ectoderm thus defined was loosened from the underlying mesoderm, and the organ removed from below. In the second and more satisfactory method, a single incision was made, dorsal to or immediately over the thickening, the tubule raised upward from below, pulled outward, and excised. In loosening the nephrostomal surfaces of the funnels, as much of the tissue in a dorsomedian direction was removed as seemed possible without disturbing the splanchnopleuric wall, since in this region the glomerulus normally arises.

Larvae in which the triple-incision method was used healed much more slowly than those to which the second method was applied, since contraction of the cut edges left a gaping wound much greater in extent and permitted more oozing of the yolk. On the other hand, when only one cut was made, a critical inspection of the excised tubules was necessary to make sure of total removal. In the majority of cases the incision is entirely healed at the end of an hour and a half.

Effect of bilateral removal

Most conspicuous of any of the postoperative conditions resulting from bilateral excisions was a pronounced edema, particularly in the anteroventral region. It is well known that edema of the amphibian embryo commonly occurs as the result of a variety of causes. Narcotized embryos reared in a solution of acetone-chloroform (chloretone), though structurally normal in other particulars, show a slight edema and pericardial effusion as a result of weakened heart action (Harrison, '04). More pronounced abnormalities result when early developmental stages are exposed to the heat of direct sunlight, or may be induced experimentally by exposing the embryos to radium rays (O. Hertwig, '11). McClure ('19), in his recent work on edema in anuran larvae, draws attention to the "less extensive tubular complex which normally occupies a dorsolateral position in the pronephros, and into which the nephrostomal canals directly open," and the "tubules which normally constitute the greater portion of the kidney and which occupy a medial and ventral position." From a study of the histological conditions existing in edematous frog larvae, he concludes that there is a functional as well as a morphological difference between these two regions, for in all of the embryos in which edema had become apparent, the ventrolateral tubules were either entirely absent or but poorly developed. From this he argues that deficiency in the ventrolateral tubules alone may be the cause of edematous conditions.

In bilaterally operated Amblystoma larvae, the swelling which first appeared in the region of the wounds progressed gradually forward, the pericardial cavity soon becoming enlarged (figs. 7
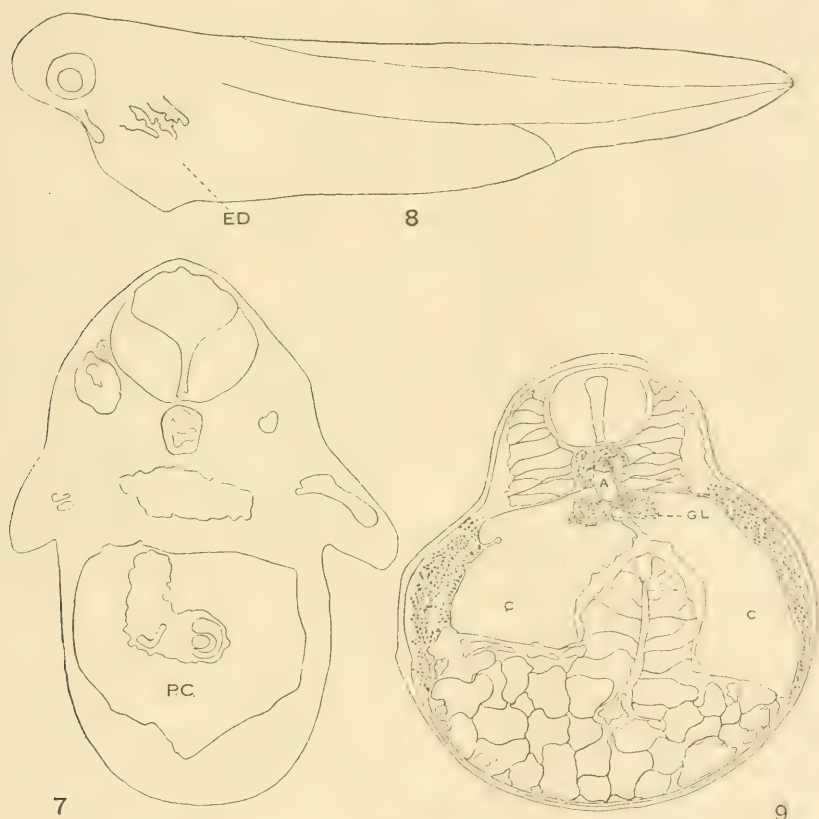
Figs. 7 and 8   Section and entire sketch of embryo, from which both kidneys had been removed, to show pericardial effusion.   Figure 7, section through pericardial (*p.c.*) region.   Figure 8, camera drawing of embryo, showing swelling in the heart region.

Fig. 9   Section through an embryo from which both kidneys had been removed. Both glomeruli (*gl*) are present, extending out into the enlarged coelomic cavities (*c*); *A*, aorta.

and 8).   Later the fluid caused a pronounced distention of the abdominal cavities (fig. 9), and in extreme cases the gills also became swollen and distorted.   Slowing or entire absence of circulation accompanied this condition and sloughing of the

ectoderm was not infrequent. Microscopic examination of sections through edematous embryos showed the tissues of the body to be in various stages of degeneration. Pressure of the accumulated fluid often forced the intestine ventrally or to one side and the fibers of the muscle plates were separated by large vacuoles. The muscle fibers themselves also became vacuolated, and in extreme cases the whole region was reduced to a spongy mass of irregular fibers with scattered nuclei.

Although the splanchnopleural mesoderm which gives rise to the glomeruli had been left intact during the operations, the question nevertheless arose as to whether normal conditions of development would obtain for these organs in the absence of other parts so closely allied with them. Sections made through the operated region in embryos killed four days and six days after double excision showed capillary tufts extending out into the much-dilated coelomic cavity (fig. 9, *gl*). The tubular region cannot, therefore, be considered to exert any influence in the nature of a formative stimulus on the development of the glomeruli, since these parts of the system arise quite independently. Furthermore, the presence of the glomeruli in these operated cases would tend to strengthen the view supported by McClure ('19) that the glomerular filtrate, given off directly into the coelomic cavity, collects here in excess, producing the typical edematous condition already described. These embryos also showed well-developed anterior and posterior funnels, extending laterally into the regions from which the tubules had been removed, and ending blindly there. Segmental ducts were present, in some cases the lumina being flattened dorsoventrally, in other instances the cells of these tubules showing marked signs of atrophy. The condition of the funnels and segmental ducts after extirpation of the kidney will be dealt with more fully in connection with the question of unilateral removal.

Efforts were made to bridge over the interval between the operations and the beginning of functional activity of the mesonephros. The first means applied was that of pricking the body wall as soon as abnormal distention was evidenced. The larvae

were immersed in 0.4 per cent NaCl in an attempt to balance the loss of essential salts through the escape of the glomerular filtrate. Although slightly stimulated heart action resulted, probably due both to the stimulus of operation and to relieved pressure in the pericardial cavity, only temporary benefit was derived in this way, for, with the accumulation of new fluid, the former pathologic condition was restored, and death followed after a short interval. It is quite possible that if a sufficient number of experiments were made, varying the constituents of the solution in which these larvae were kept, a satisfactory medium might be found for prolonging the life of these animals. A determination of the optimum salt percentages of such a solution has not yet been undertaken.

The second means employed was the transplantation of the pronephric rudiment to the region of the mesonephros. It was by this means possible to test the capacity for reestablishment of function, through union with the segmental duct. In a series of thirty embryos, the right head kidney, without the ectodermal covering, was placed under the skin farther back on the same side. Twenty-four hours later, the left pronephros was removed from those embryos which had responded well to the first operation and were apparently recovered. The transference and proper orientation of the kidney in these operations was easily accomplished and the wounds healed entirely in the usual short time, but the general edematous condition common to embryos on which only the bilateral excision had been performed subsequently developed. With the exception of a few which were preserved and sectioned at the end of a week, all of this series died within twelve days, showing no indication of resumption of function by the transplanted tubule. The transplanted tubule still retained its identity, although as a general rule the cells were pressed together in a solid mass, and only in a few instances a distinct lumen was visible. Removal of the pronephros resulted here in the partial or total atrophy of the segmental duct, to which attention will be called in greater detail below. However, in cases where the transplanted tubule had been placed in the immediate region of the segmental duct, no connection was

restored between these two components, nor did the duct, posterior to the transplant, give any evidence of functioning.

A slightly different method was applied in a third series of experiments. The pronephros was not alone transplanted, but was taken together with the overlying ectoderm, the surrounding mesoderm, and even small portions of the ventral myotomal walls. This transplant was procured from another animal, and was transferred into a previously prepared incision and held in place until healed. On the next two successive days the left and right pronephric rudiments were removed. No appreciable
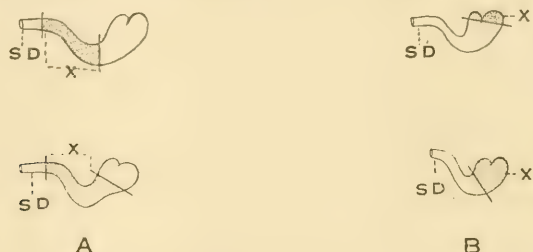
Fig. 10, A and B Diagrams to show the location and extent of operations made in removal of different segments of the embryonic pronephros. In A the segment removed, x, was a part or the whole of the U-shaped tubule anterior to the segmental duct. In B the segment removed, x, consisted of a part or the whole of the rudiment of the funnels.

difference was noted in the ensuing condition of this series, and it is safe to conclude that under these circumstances the excised tubule is unable to readjust itself and function in its new location.

Interruptions to the development of the pronephros by a less radical operation also go to strengthen the belief that the regenerative capacity of the kidney tubule is either very limited or very slow in taking place.[6] In a number of embryos from which one pronephros had been extirpated, a small portion of the opposite rudiment was also excised. The segments removed (x) were at two levels, as designated in figure 10, A and B. The funnels were undisturbed in one group (A), a short piece of the

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[6] This does not apply to the coelomic epithelium which lines the nephrostomal opening, as will be shown in a later section.

first loop being removed, while in the second group (B), the funnel rudiment was cut off just anterior to the first bend. Of the twenty specimens used, all exhibited symptoms identical with those induced by bilateral excision.

The extent of regeneration which would occur in a defective tubule if the opposite kidney were allowed to remain intact is still a matter for investigation, but it is not improbable that a given portion of a tubule may possess a prospective potency which would insure the restoration of an excised section. However, in dealing with an organ where the demand for functional activity follows so closely upon this disturbance of normal condition, the requisite time for readjustment may be the factor lacking. Accumulation of excretory fluid may inhibit the regeneration which might be the normal consequence, if excretory activity were maintained by an undisturbed kidney. A fundamental difference thus places excision of the kidney in a category apart from the majority of regeneration or transplantation experiments upon the amphibian embryo which have been reported up to the present, for the effects consequent on extirpation and transfer of limb rudiments, optic vesicles, or nasal pits, though abnormal, are not of a nature to interfere with any of the vital functions of the embryo.

UNILATERAL EXCISION OF PRONEPHRIC RUDIMENTS

Conclusive evidence having been obtained as to the essential nature of the pronephros in the life of the embryo, a further study of the correlation of the development of this organ with that of the other components of the excretory system was then undertaken.[7] Unilateral excision of the pronephric rudiment served.as a practical means to this end.

The technique of operating has already been discussed in the previous section, but a word of explanation is necessary regarding the controls used in this series. Since a more or less pronounced retardation in growth was the unavoidable consequence

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[7] As has been previously stated, the glomerulus was found to develop normally even in the absence of the pronephric coil.

of such operations, the controls were always more advanced than the operated embryos, so that, for the comparison of the excretory organs, others had to be selected as described below (p. 373).

Postoperative effect on the embryo as a whole

Every outward evidence of successful readjustment to the new conditions imposed was shown by the operated larvae. Adverse symptoms, such as edema and general sluggishness, were absent, and, barring the slight retardation already mentioned, normal progress continued, except in those cases in which the limb bud was disturbed or entirely removed. The ectodermal surface which showed a slight concentration of pigment in the initial stages of wound healing gradually became indistinguishable from the surrounding regions, and differed from the opposite side only in the absence of the distention caused by the underlying pronephric coils.

Effect of unilateral excision on the remaining pronephros

The pronephros remaining after removal of one head kidney obviously takes over the function of excretion usually performed by the two organs. Beginning with the fourth day after excision, operated embryos were killed for observation each day for a period of two weeks. Sections showed distinct changes in the several remaining components of the excretory system, particularly in the head kidney functioning alone, the size of which was indicative of a marked compensatory hypertrophy. Since the controls taken from the same egg mass and carried along under the same conditions as those to which the operated forms were subjected invariably showed on sectioning a more advanced stage of development, the first step in determining the nature and extent of the change in the operated kidney was the establishment of a criterion for comparison of an operated with a normal embryo. An operated individual (PN 7) was chosen as a typical case and a large number of normal larvae of apparently the same age was examined to obtain

one in which the stage of development was identical. In many embryos where superficial features, such as length, breadth, and condition of limb and gill rudiments, were the same as those of PN 7, it was found on sectioning that the internal organs varied widely in degree of development. The normal larva finally selected (PN 7 d) tallied[8] not only in external measurements, but showed the several internal organs (retina, lens of eye, digestive tract, etc.) to be in a stage corresponding to those of PN 7.

As a further check against the possibility of error in the choice of a normal duplicate, a second duplicate was chosen, and the respective volumes of the kidneys of the two roughly compared by the following method:[9] On drawing-paper of uniform thickness the serial sections of the entire kidney of PN 7 d and of the second duplicate (PN 7 d, no. 2) were projected and the lumen of the tubule outlined. The drawings of each kidney were then carefully cut out. No attempt was made to assemble them in the form of a model, but the weight of the paper used for each was taken as a standard for comparison. The weight of PN 7 d was 2.35 grams and that of PN 7 d, no. 2, 2.26 grams, giving a difference of only 0.09 gram, or about 4 per cent—a variation so small as to be considered negligible. The larger normal kidney (PN 7 d) was used for comparison with the hypertrophied one in order to lessen the possibility of exaggerating the difference between the two.

After the normal duplicate (PN 7 d) had been selected, several methods were open for the determination of the nature and degree of the hypertrophy of the remaining pronephros in the embryo from which the organ on one side had been removed.

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[8] The slight variation would tend rather to minimize the contrast than to accentuate it, since, if either, PN 7 d is the more advanced.

[9] In connection with the review of Kittleson's paper (see previous reference), I find that somewhat the same method was employed by him in his estimation of the relative surface areas and weights of the kidneys of rats. The weight in grams was reduced to square centimeters by estimating the average area in square centimeters of one gram of paper, and from this the total volume of the kidney was estimated.

Wax models of the unoperated right pronephros of PN 7 and
the corresponding organ in PN 7 d were constructed by means
of the Born method, at a magnification of 200 (figs. 18 to 21).
Unlike the normal model, to which reference has already been
made in an earlier section, these two are reconstructions of the
lumen of the tubules without enclosing walls. The model of
the kidney of the operated embryo not only showed a consider-
able increase in the thickness of the tubule as contrasted with
that of the normal, but its length is also appreciably greater.
This was determined by taking the average of five measure-
ments. A flexible but inelastic cord was pinned along the sur-
face of the wax for its entire course, and its length thus recorded.
On each measurement the cord was pinned along a different sur-
face, so that the data would be of a representative nature. For
the model of the normal kidney the average length was found
to be 155 cm., at a magnification of 200, with a probable error
of ±0.181: for PN 7, 188 cm., at a magnification of 200, with a
probable error of ±0.155, showing an increase of 21 per cent
over the normal condition.

Microscopic examination of the tubules showed the walls in
the normal organ (PN 7 d) to be relatively thick and made up of
cuboidal cells. The hypertrophied tubules (PN 7) were thinner
walled proportionately, the cells often flattened and elongated,
and the lumen strikingly larger than that of the unoperated
specimen[10] (figs. 22 and 23). Outline drawings were made of
the outer and inner boundary of the walls of the hypertrophied
and of the normal tubules at a magnification of 600 (figs. 11 and
12). A non-elastic cord was then pinned at frequent intervals
along the inner lines, removed and measured, and the circum-
ference obtained. Five different sections were used at different
levels in each case, and in each section from three to five tubules
were measured, making a total of twenty-one measurements for
each kidney. The average measurement obtained for PN 7 d

[10] In a series of experiments reported by Detwiler ('18), the pronephros was
often carried along in the transplantation of the limb rudiments. The enlarge-
ment of the undisturbed pronephros and its contrast with the normal condition
may also be seen on examination of his plates (Jour. Exp. Zoöl., vol. 25, 1918,
pl. 3, figs. 18 and 19).

was 78 mm. (0.13 mm. in actual size), as contrasted with 130
mm. (0.216 mm. in actual size) for PN 7—a fact suggesting the
large percentage of increase in functional capacity of the two
kidneys determined and described below. From these projec-
tions also the thickness of the walls was estimated by taking the
average of sixty measurements in each kidney. The average
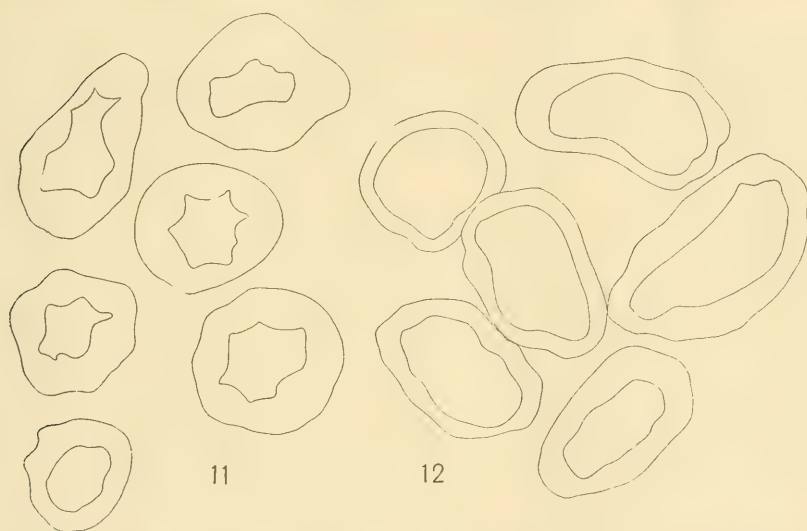thickness of the wall of the normal kidney is 10.9 mm. at a mag-

Fig. 11, PN 7 d   Normal tubules with thick walls, and cells bulging into the
lumen. × 240.

Fig. 12, PN 7   Hypertrophied tubules with large lumens and thinner walls.
× 240.

nification of 600, or 0.0181 mm. in actual measurement. For
the hypertrophied kidney, the average thickness is 7.8 mm. at
a magnification of 600, or 0.013 mm. in actual measurement.

Having the length of the two kidneys, determined from the
models, and considering each kidney as a simple cylinder, with
the average measurement just obtained as its circumference
and the length as its height, the areas of the inner surfaces in
each were computed. In the normal kidney the area of the
secreting surface was found to be 1.007 sq. mm. as contrasted

with 2.037 sq. mm. in the compensating organ—an increase of more than 100 per cent (table 1).

A comparison of the volumes of the cells making up the walls of the two kidneys is likewise of great importance in determining the nature of the response brought about by a unilateral operation. Although in the calculation of the surface area of the tubules it seemed sufficiently accurate to regard them as cylinders with the average circumference as their boundary, it did not seem possible to apply such a geometrical method in estimating the volume of the walls, for the cells (figs. 11 and 12), especially in the normal kidney, often bulge out into the lumen, making this, as seen in projected outline, very irregular. The method already described in the selection of a normal model was again

TABLE 1

Showing the area of the inner or secreting surfaces of the normal kidney, PN 7 d, and the hypertrophied kidney, PN 7

SERIES NUMBER	INNER CIRCUMFERENCE ($\times$ 600)	OUTER CIRCUMFERENCE ($\times$ 600)	LENGTH ($\times$ 600)	AREA OF INNER SURFACE ($\times$ 360,000)	ACTUAL AREA OF SURFACE
	cm.	*cm.*	*cm.*	*sq. cm.*	*sq. mm.*
PN 7 d (normal)........	7.8	13.6	465	3627	1.007
PN 7 (hypertrophied)....	13	18.6	564	7332	2.037

used (p. 373). Paper of uniform weight was obtained, and to insure further accuracy, section number one of the normal kidney was projected on one half of a sheet, section one of the hypertrophied organ on the same sheet of paper, and so on through the series. The walls of the two kidneys were then cut out, and the aggregate of each weighed separately. The paper representing the walls of PN 7 d weighed 20.39 grams that of PN 7, 33.4 grams, showing an increase of 63 per cent in the weight of the latter. Since the weight in this case is in direct proportion to the volume, the hypertrophied kidney may then be considered as showing an increase of almost two-thirds beyond the normal. A count of the nuclei in each kidney, all the sections of which were cut at 10μ, shows a small percentage (16 per cent) of increase in the number of cells found in the larger kidney,

there being 1950 nuclei in PN 7 d and 2252 in PN 7.   Although this may indicate a certain amount of hyperplasia, that is, increase in actual number of cells present, it is not large enough to account for the great percentage of increase in both secreting surface and in volume.   This increase must be mainly attributed to actual hypertrophy or enlargement of the cells already present.

Effect of unilateral excision on the glomerulus

A study of the condition of the glomeruli subsequent to double excision was necessarily limited by the early death of the embryos. The occurrence of the glomerulus in these cases has already been noted.   On examining larvae from which only one pronephros had been removed, both glomeruli were found to be invariably present.   The one on the operated side, however, exhibited less uniformity in size and shape than the normally functioning one. In some cases the outer layer of the glomerulus and the epithelial lining of the body wall had coalesced, this being the case not only on the operated, but on the normal side as well.   On the operated size, however, the distance to be bridged is very greatly increased, since the absence of the pronephric swelling increases the width of the coelomic cavity there (fig. 13).

Since the development of this part of the kidney unit is quite independent of the presence of the tubular portion, its functional activity can be counted on to continue undisturbed.   No hypertrophy in glomerular structure, then, would be anticipated, nor was such found to be the case, for the structural conditions existing allow free passage of the filtrate ventrally from one side to the other through the coelomic cavity.   Filtration continues on both sides, the demand for increased physiological activity falling only on the tubules of the unoperated side.

Effect of unilateral excision on other components of this system

Removal of one head kidney has a widely varied effect on the formation of the segmental duct on the operated side.   The process of development of the non-functioning ducts took place

irregularly, and, in general, only to a limited extent. As shown in table 2, every gradation occurred, ranging from a condition in which the lumen, though small and flattened dorsoventrally, appeared throughout the entire length (A 51, 5 days) to a condition where only the occasional presence of a few degenerating cells indicated the location of the atrophied rudiment (A1, 10 days).

Fig. 13, PN 2 Showing the increased width of the glomerulus (*gl*) on the operated side. Description in text. × 40.

Increased activity of a single kidney also has a definite effect on the segmental duct of that side. Cross-sections of the duct of an individual with unilateral operation, when compared with either of the ducts of a normal larva of the same age, show a marked increase in diameter.

Although in the excision of the pronephric rudiment great care was taken to remove as much of the somatopleural mesoderm as seemed feasible without disturbing the underlying tissues, an examination of the operated embryos showed that a large num-

ber possessed well-developed anterior and posterior nephrostomes ending blindly (figs. 14 and 15 and table 2). This would indicate that the adjacent coelomic endothelium possessed the

Fig. 14, PN 2 Showing double anterior funnel (d.f.). × 30.
Fig. 15, PN 7 A, regenerated anterior funnel (a.f), on the operated side. B, regenerated posterior funnel (p.f) on the operated side. × 30.

capacity for regeneration of this portion of the organ, even though, as we have already seen, this property is not shown by the tubules themselves. Of the fifteen embryos tabulated

TABLE 2

Showing condition of ducts, nephrostomes, and glomeruli in embryos from which one pronephros has been removed

SERIES NUMBER	AGE	DUCT ON OPERATED SIDE	DUCT ON UNOPERATED SIDE	NEPHROSTOMES	GLOMERULI
	days				
A 54	4	Canalized at intervals, flattened	Round, canalized, definite	Anterior present, posterior absent	Both present
A 51	5	Small, canalized, flattened	Large, round, canalized	Anterior present, posterior present	Both present
A 51	6	Small, flattened, atrophied posteriorly	Large, round	No anterior, posterior present	Both present
A 54 no. 1	7	Small, canalized	Large, round	Anterior present, posterior absent	Both present
A 54 no. 2	7	Present anteriorly atrophied posteriorly	Large, round	Anterior present, posterior present	Both present
A 54 no. 3	7	Present anteriorly, atrophied posteriorly	Large, round	Anterior absent, posterior present	Both present
A 8	9	Small, slightly canalized	Large, round	Small anterior, no posterior	Both present
A 1	10	A few degenerating cells	Large, round	Anterior present, posterior absent	Both present
A 1	11	Small, irregularly canalized	Very large	Anterior present, posterior present	Both present
PN 2	17	Very small but canalized posteriorly	Large, round	2 anterior, 1 posterior	Both present
PN 3	29	Small, discontinuous anteriorly	Large, but flattened anteriorly	Anterior present, posterior present	Both present
PN 4	15	Very small, (whole mount)	Large, (whole mount)	No anterior, posterior present	Both present
PN 5	21	Small (whole mount)	Large, (whole mount)	No anterior, posterior present	Both present
PN 6	21	Small anteriorly, central portion discontinuous	Flattened anteriorly	Anterior present, posterior present	Both present
PN 7[1]	9	Small anteriorly, none posteriorly	Large, round	Anterior present, posterior present	Both present

[1] For PN 7, 9 days, see figs. 18 and 20.

(table 2), twelve had well-formed anterior, and twelve had posterior nephrostomes. In one instance (PN 2, 17 days) the anteror nephrostome had doubled, suggesting the three pronephric openings normally found in anuran larvae (fig. 14 and table 2).

A study of the development of the mesonephros in operated animals will be the subject of further work.

EFFECT OF REMOVAL OF THE HEART ON THE DEVELOPMENT OF THE GLOMERULUS

In a series of experiments dealing with the effect of removal of the heart on certain other organs of the embryo, Doctor Harrison removed the rudiment of the heart in larvae of stages 29 to 30. Through his kindness, these embryos were made available to me for a study of the effect produced on the glomeruli. With the incoming of new material (March, 1920), these cases were further augmented by additional experiments.

The glomerulus in Amblystoma punctatum, as has been stated in an earlier section, normally begins to differentiate from cells of the splanchnopleural wall below and at each side of the aorta, in stage 36. Within these clusters of cells vacuolated areas soon appear, and in a short time connect with the aorta.[11]

In embryos from which the heart has been removed before any contraction of the cardiac muscles occurred, the initial development of the cell groups is normal. However, as the connection with the aorta is established, the more or less compact nature of the tufts can no longer be maintained, but from pressure of the blood plasma which has collected in and is distending the bloodvessels, the vacuolated centers of the glomeruli are torn apart. As this accumulation of fluid increases, the outer walls of the tufts become more and more flattened, and consequently less easily distinguishable from the wall of the aorta, with which they are still continuous, finally losing their identity as separate organs. It is of interest, however, to note their early formation under these circumstances as additional proof of their independent power of development.

[11] A detailed description of this process together with plates is given by Field, '91 (pl. 1, figs. 8, 9, 10; pl. 6, figs. 48, 49, 50, 52, etc.).

SUMMARY AND CONCLUSIONS

From a study of the results obtained after bilateral and unilateral extirpation of the head kidney and of the heart rudiment of Amblystoma larvae, the following conclusions may be drawn:

1. Conditions ensuing on bilateral removal of the pronephros show clearly that this organ is necessary to the life of the embryo, although the presence of one pronephros suffices to keep the organism alive and in a healthy condition. All embryos from which both head kidneys had been extirpated died within from eight to twelve days, evidencing during that interval weakened heart action, edema, and effusion into the pericardial and abdominal cavities. Pricking the body wall to relieve the edematous condition proved ineffective.

2. Double extirpation does not affect the normal development of the glomeruli. These appear in embryos killed four days after the operation.

3. The pronephros remaining after the removal of one head kidney takes over the function of excretion usually performed by the two organs, and, concomitant with the increased physiological activity, presents marked morphological changes.

4. The adjustment consequent on unilateral removal consists not in the regeneration of the lost part, but in compensatory hypertrophy of the remaining organ, a response which has long been known to occur in the adult kidney and in other glandular organs, both paired and unpaired.

5. The area of the secreting surface in the hypertrophied kidney shows an increase of over 100 per cent when contrasted with the normal (2.037 sq. mm.; 1.007 sq. mm.).

6. The cubic content of the mass of cells constituting the hypertrophied kidney as shown by their relative weight is increased 63 per cent above the normal.

7. The length of the tubules shows an increase of 21 per cent.

8. The number of nuclei in the hypertrophied kidney exceeds that of the normal by 16 per cent, due to the occurrence of a small amount of hyperplasia.

9. In single, as well as in double, extirpations, the glomerulus develops normally in the absence of the pronephric tubules.

10. Anterior and posterior nephrostomal funnels are regenerated from the coelomic endothelium in a large proportion of operated embryos.

11. The segmenta' duct on the operated side shows great variation in development, ranging from a condition in which the lumen, though small and flattened dorsoventrally, appears throughout the entire length, to a condition where only the occasional presence of a few degenerating cells indicates the location of the atrophied duct.

12. Increased activity of a single kidney also has a definite effect on the segmental duct of the same side. Cross-sections of the duct of an individual with unilateral operation, when compared with either of the ducts of a normal larva of the same age, show a marked increase in diameter.

13. In embryos from which the heart rudiment has been removed in a very early stage, the initial development of the glomeruli is normal. Subsequent distention of the aorta tears the cells apart and they soon lose their identity as lateral capillary tufts.

LITERATURE CITED

BYRNES, ESTHER F. 1898 On the regeneration of limbs in frogs after the extirpation of limb rudiments. Anat. Anz., Bd. 15.

DETWILER, S. R. 1918 Experiments on the development of shoulder-girdle and the anterior limb of Amblystoma punctatum. Jour. Exp. Zoöl., vol. 25, no. 2.

FIELD, H. H. 1891 The development of the pronephros and segmental duct in Amphibia. Bull. Mus. Comp. Zoöl. Harvard. Coll. 21.

FÜRBRINGER, M. 1878 Zur vergleichenden Anatomie und Entwicklungsgeschichte der Exkretionsorgane der Vertebraten. Morph. Jahrb., Bd. 4.

GALEOTTI, G., UND VILLA-SANTA, G. 1902 Über die kompensatorische Hypertrophie der Niere. Beitr. z. path. Anat. u. allg. Pathol., S. 121–141.

HARRISON, R. G. 1904 An experimental study of the relation of the nervous system to the developing musculature in the embryo of the frog. Am. Jour. Anat., vol. 3.
    1918 Experiments on the development of the fore limb of Amblystoma, a self-differentiating equipotential system. Jour. Exp. Zoöl., vol. 25.

HERTWIG, O. 1911 Die Radiumkrankheit tierischer Keimzellen. Arch. mikr. Anat., 2te Abt., Bd. 77.

HOWLAND, RUTH B. 1916 On the effect of removal of the pronephros of the amphibian embryo. Proc. Nat. Acad. Sc., vol. 2.

KAMMERER, P. 1905 Über die Abhängigkeit des Regenerationsvermögens der Amphibienlarven von Alter, Entwicklungsstadium und spezifischer Grösse. Roux's Archiv, Bd. 19, 2. Heft.

KITTLESON, JOHN A. 1920 Effects of inanition and refeeding upon the growth of the kidney of the albino rat. Anatomical Record, vol. 17.

KOCHS, W. 1897 Versuche über Regeneration von Organen bei Amphibien. Arch. mikr. Anat., Bd. 49.

LEVI, GIUSEPPE 1905 Lesioni sperimentali sull' abbozzo urogenitale di larve di Anfibi e loro effetti sull' origine delle cellule sessuali. Roux's Archiv, Bd. 19.

McCLURE, C. F. W. 1919 Experimental production of edema in larval and adult anura. Jour. Gen. Physiol., vol. 1, no. 3.

MÜLLER, JOHANNES 1829 Ueber die Wolff'schen Körper bei den Embryonen der Frösche und Kröten. Meckel's Arch. f. Anat. u. Physiol, Jahrg. 1829, S. 65–70. Taf. III.

PRICE, G. C. 1910 The structure and function of the adult head kidney of Bdellostoma stouti. Jour. Exp. Zoöl., vol. 9.

SACERDOTTI, C. 1896 Ueber die compensatorische Hypertrophie der Nieren. Virchow's Archiv, Bd. 146, S. 267.

SWINGLE, W. W. 1919 On the experimental production of edema by nephrectomy. Jour. Gen. Phys., vol. 1, no. 5.

WOLFF, M. 1900 Die Nierenresektion und ihre Folgen. Berlin. Hirschwald.

WITTICH, VON 1852 Beiträge zur morphologischen und histologischen Entwickelung der Harn- und Geschlechtswerkzeuge der nackten Amphibien. Zeitschr. f. wiss. Zool., Bd. 4, S. 125–167, Taf. X, XI.

PLATES

ABBREVIATIONS

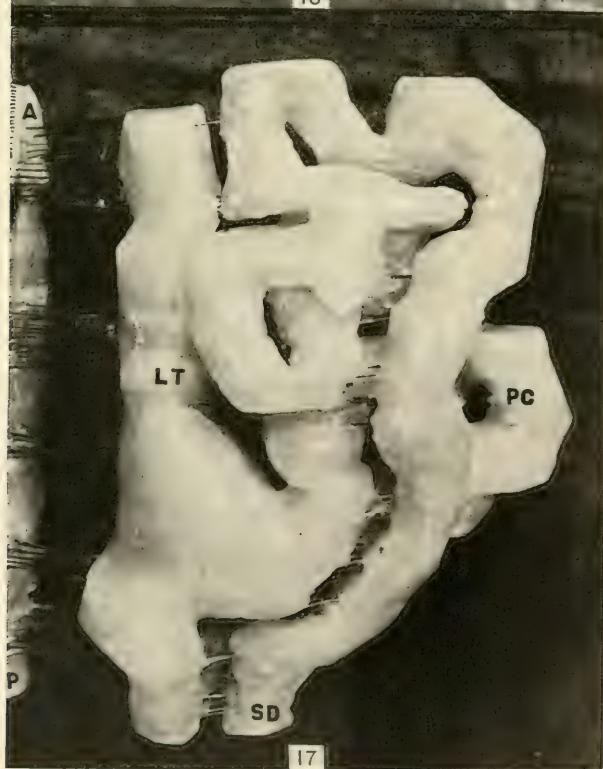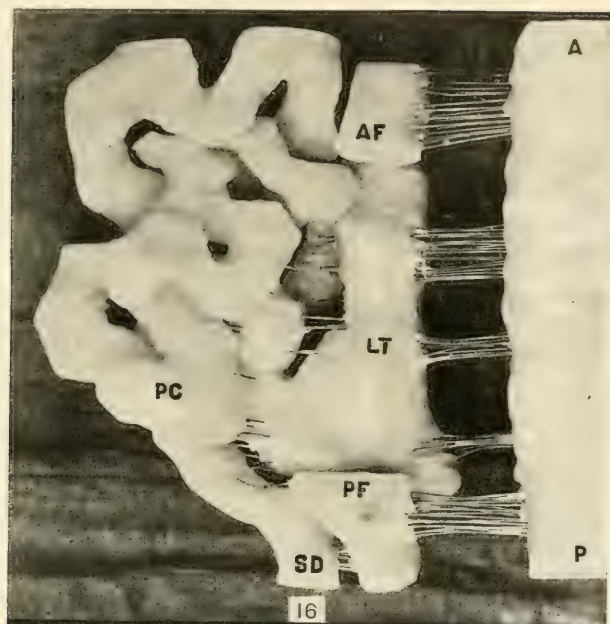*A*, anterior

AF, anterior funnel

LT, longitudinal tubule

P, posterior

PC, pronephric coil

PF, posterior funnel

SD, segmental duct

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PLATE 3

20   Model of hypertrophied pronephros, dorsolateral view (PN 7).

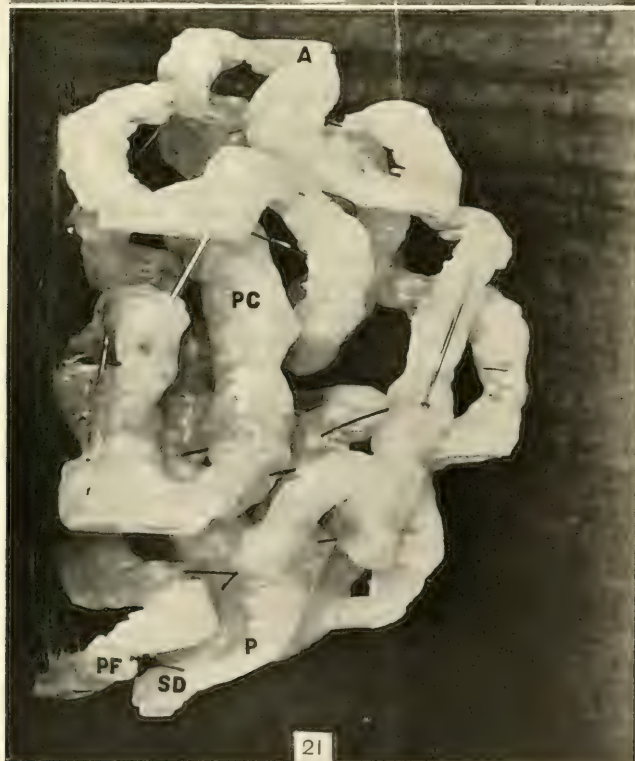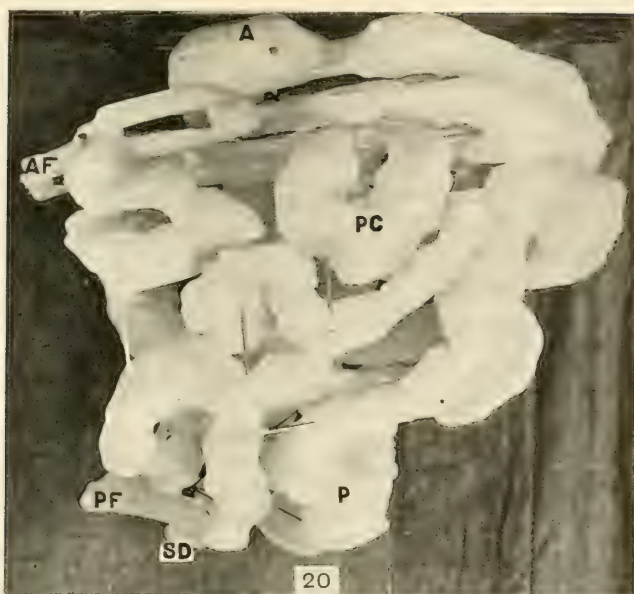21   Model of normal pronephros, of same age as figure 20, dorsolateral view (PN 7, d).

Resumen por los autores, W. A. Kepner y W. Carl Whitlock.
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Reacciones alimenticias de Ameba proteus.

En este organismo existen dos tipos generales de reacción en
presencia de los alimentos: (a) Cuando la presa no puede escapar
la amiba la rodea estrechamente; (b) Cuando puede escapar la
amiba corta su retirada envolviéndola con sus pseudópodos, y
entonces la presa queda capturada. Estos dos tipos de reacción
alimenticia no son fijos, sino que varían notablemente. Al
reaccionar en presencia de un objeto que se mueve general-
mente en un plano horizontal, la amiba rodea la presa primero
en este plano, y después corta su retirada en un plano vertical.
Generalmente una reacción tiene lugar mediante cooperación
del ectoplasma y el endoplasma, aunque el primero por sí solo
puede llevar a cabo una reacción del segundo tipo. Tanto el
ectoplasma como el endoplasma son muy contráctiles cuando
las condiciones lo exigen.

La fragmentación de un animal como Paramoecium en dos
pedazos es primariamente un proceso físico y no químico, y la
digestión comienza después que la presa ha sido fragmentada.
El proceso de la ingestión del alimento es reversible. Alimento
medio ingerido, casi ingerido o completamente ingerido puede
ser expulsado. Las reacciones de Ameba difieren de los fenó-
menos físicos y químicos en que son cualitativas más bien que
cuantitativas y se llevan a cabo en interés del organismo.

FOOD REACTIONS OF AMEBA PROTEUS

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SIX PLATES (TWENTY-ONE FIGURES)

The observations presented in this paper are selected from many that have been recorded by members of the staff of this laboratory. Some of the observations were taken from specimens in Petri dishes, some in uncovered drops, still others under cover-glasses, while many were secured from amebas that had been kept in hanging drops until time was available for making observations.

We have found Ameba proteus reacting to two types of food. The first type embraces the following forms: desmids, Mougeotia, quiet Oscillatoria, encysted Chlamydomonas, and bacterial gleas; while the second group of food bodies comprises flagellates like Chilomonas, Peridinium, and Euglena, ciliates like Paramecium caudatum, Colpidium, Cyclidium, and rotifers. The first of these groups of food objects is characterized by being nonmotile, the second group by being motile. Some of the nonmotile objects give off oxygen, while others give off carbon dioxide. The same may be said of the motile group; it, too, may be subdivided into the forms that give off oxygen and those that yield carbon dioxide to the surrounding medium. Therefore the most conspicuous difference between these two groups of food is the non-motility of the first group and the motility of the second group.

Correlated with this conspicuous difference between the two types of food of Ameba there is a two-fold food reaction on the part of these rhizopods. Ameba's conduct toward non-motile food is much less complex than its conduct toward motile food. The less complex type of reaction is concerned with ingesting forms that do not set up currents in the surrounding water and

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that do not present the contingency of escape. The more complex type of food reaction of Ameba is concerned with the capture of forms that set up currents in the surrounding water and that do present the contingency of escape. It was interesting to us to find that Leidy ('79) had shown in his figure 5, plate 1, two Urocentra captured according to our second type of food reaction, while a green plant cell was ingested apparently by the first type of food reaction.

EXAMPLES OF THE FIRST TYPE OF FOOD REACTION

a. *Objects that yield oxygen to the water*

On March 15, 1919, we found that in a Petri dish there were many filaments of Oscillatoria that were quite quiet. None of these filaments were to be seen moving as Oscillatoria filaments frequently do. An ameba had ingested one end of one of these quiet filaments (fig. 1). In endeavoring to take this specimen from the Petri dish on to a slide, the capillary canula of the pipette dragged over the free end of the algal filament in such manner as to tear the ameba from the substratum and turn it through about 180 degrees. The ameba was now given time to fix itself again to the bottom of the dish. The free end of the filament was then pushed against with the canula of the pipette. This time instead of the ameba's being torn from the bottom of the dish, the part of the ameba's body that surrounded the filament was bent from position *a* to position *b* (fig. 2.) The ameba was now drawn up into the capillary pipette and transferred to a hanging drop. The compound microscope showed that, despite this relatively rough handling, the ameba yet held on to the filament of Oscillatoria. Within two minutes after the coverglass was placed over the glass ring, the Oscillatoria was egested. Soon after this the ameba again ingested one-third of the length of the filament and then threw it out a little later. The ameba a third time set to ingesting the plant. When about one-fourth of the filament was within the body of the ameba, a paramecium collided sharply with the projecting end of the alga at right

angles, and the ameba then gave up its efforts to lay hold of this food. Throughout all of the time that the ameba was working on this rather long filament of Oscillatoria it had within its body a filament of Oscillatoria that was 30μ long when first seen. Within the course of our observation, this ingested filament was broken up into three pieces, one 10μ, one 5μ, and one 15μ.

After we had secured several observations showing that the ameba laid hold of quiet Oscillatoria filaments tightly, we called in some of our colleagues in this laboratory to make observations. Six others verified our results by making similar observations. The most conspicuous of these corroborative records was made by Dr. I. F. Lewis. He was given an ameba that had ingested an end of a very long filament, indicated as broken off in figure 3. He took a fine glass rod and bent the plant to contour b (fig. 3), at which point the tension of the alga caused it to spring back as a straight rod. "Twenty big bends, some like this, others different, were made as the ameba gradually lost its hold."

No such large filaments have been ingested wholly. The ameba sometimes travels from end to end along such long objects, sometimes making several trips, and then leaves the food behind. Frequently small fragments have been seen in different stages of digestion within amebas (fig. 5, O). It would seem that the ameba seeks the planes of fission of the Oscillatoria filaments to break off fragments for food. Such may not be the case, however, for we have seen an ameba travel along a Mougeotia filament in a similar manner, and there are no fission planes in Mougeotia. No Mougeotia filaments or fragments were ever seen completely ingested. Large desmids were also ingested in part and then rejected. On one occasion, January 28, 1919, two amebas began to ingest opposite ends of a large Penium synchronously. The lower half of the desmid was ingested within twenty minutes by one of the amebas. During this period the upper ameba ingested about one-third of the desmid. Both amebas were closely embracing the plant, but they eventually rejected the object by withdrawing from it. Small desmids have been observed by us being ingested by Ameba proteus. A Chlamydomonas within its gelatinous sheath was also ingested. Nei-

ther the small desmids nor the encysted Chlamydomonas rolled before the advance of the ameba, and they too were ingested within a closely fitting food vacuole. The observation, based upon the ingestion of an encysted Chlamydomonas, makes an interesting contrast with Jennings's ('04) observation of an ameba ingesting an encysted Euglena. In the latter case the encysted alga rolled ahead of the advance of the ameba, and here Jennings saw a cup form behind the algal cysts. This contrast between our observation and that of Jennings suggests that even the type of reaction involved in ingesting non-motile objects may be modified to meet an unusual turn of events. There are some non-motile food objects which give off carbon dioxide. Of these, bacterial gleas form common examples. February 16, 1918, Dr. R. D. Mackay observed an ameba glide over a glea. As it was about to leave the glea, two embracing pseudopods were sent out about the bacterial mass. These pseudopods lay close up to the sides of the rounded mass and eventually constricted a small portion from the glea as the enclosing pseudopods began to converge (fig. 4, a and b).

In the above reactions we have the ameba responding to non-motile objects that gave off either oxygen or carbon dioxide. In reacting to this class of food, the amebas seized the objects in an intimate embrace.

The following constitute a list of motile objects to which Ameba proteus has been seen reacting: a) Euglena viridis, Peridinium, and diatoms; b) rotifers, Paramecium caudatum, Urocentrum, Glaucoma scintillans, Colpidium, and two species of unidentified ciliates, Chilomonas, Codosiga, Euglena acus, and two species of unidentified flagellates. Of this group of motile food objects, a) forms a subdivision of forms that give off oxygen, while b) forms a subdivision of forms that yield carbon dioxide to the surrounding medium.

The reaction of ameba to diatoms has been rather indefinite. The ameba seems to react to these motile plants as if they were non-motile. We have, however, obtained but two observations based upon diatoms, and in both of these cases the diatoms, while they were being intimately embraced, escaped.

Except for the diatoms, we have seen that there is a wide range of motile food bodies to which Ameba proteus displays a general type of response. The following observations have been chosen as examples of the ameba's second type of food reaction and also to display the range of adaptive modification this type of reaction may present.

The ameba seems to have a marked preference for Chilomonas paramecium. It will readily accept one of these little ciliates, though it has been feeding on a non-motile object or other motile objects. On March 19, 1919, we observed a specimen that had been feeding upon Oscillatoria. A Chilomonas swam into a bay between two stout, short pseudopods and lay in the position shown in figure 5. The ameba immediately sent two secondary pseudopods, A and B, out toward each other and behind the Chilomomas. These pseudopods met and fused; the ciliate was thus surrounded on all sides. It was next overarched by a thin sheet of ectoplasm. When all lines of retreat were thus cut off from the Chilomonas, the ameba reduced the size of the large vacuole, within which the prey had been captured, to that of the usual food vacuole. Both ectoplasm and endoplasm entered the formation of the pseudopods A and B in this reaction. This is the manner in which the enclosing pseudopods are usually constructed. But even the structure of the pseudopods may be modified to meet the needs of a peculiar situation.

In one instance we observed an ameba approach two Chilomonases in the shallow margin of a hanging drop. In this case ectoplasmic pseudopods a and a' were sent out about the Chilomonases (fig. 6). As a grew down to contour b, an overarching layer of ectoplasm, c, was formed above the prey. The internal margins thus formed eventually fused as b grew down to divide the enclosed space into two food vacuoles. The animal then moved out into deeper water. The unusual feature of this reaction is not that the overarching protoplasm is ectoplasmic, for that and the underhanging wall of the forming food vacuole are usually ectoplasmic. The unusual feature is the fact that the ectoplasm formed all sides of the forming food vacuoles. These vacuoles were thrown into the endoplasm when the animal moved

out into the deeper regions of the drop after capturing the two flagellates.

During the course of this observation it was noticed that an ameba does not of necessity react to an object that is setting up currents in the surrounding water or that is colliding with the ameba repeatedly; for before, during, and after the reaction of the ameba to the above two Chilomonases, a very active, dense swarm of bacteria plied to and fro against the side of the ameba making frequent contacts with it. At none of these contacts did the ameba react to this highly motile mass. It mattered not whether the contact were made at an angle between pseudopodia, as at A, figure 6, or at the tips or sides of the pseudopods.

A newly formed pseudopod that is taking part in the formation of a food vacuole may further react to cooperate with a part of the body proper to construct a second food vacuole. That such is the case is shown by the following example. Two Chilomonases were being surrounded by pseudopods a and a' (fig. 7). When a had grown to contour b, a third Chilomonas came up by the side of a'. In reacting to this third Chilomonas, the body proper threw out pseudopod c', while pseudopod b sent out c to meet c'. In this manner all three flagellates were captured.

On March 19, 1918, we saw an advancing pair of pseudopods, a and b, encounter a relatively large piece of foreign matter as they advanced about a Chilomonas which lay in position indicated in figure 8. At this synchronous contact of the two pseudopods the one, b, was arrested while a advanced to contours c and d, d finally fusing with the body proper. The Chilomonas was next overarched and captured.

Perhaps a more striking example of a reaction involving foreign matter is presented in our observation of an ameba ingesting a paramecium that lay in a shallow bay by the side of a large brown mass of detritus (fig. 9). The ameba was advancing in a general way toward the paramecium along pseudopods 1, 2, and 3. As it approached the ciliate, pseudopods 1 and 2 widened and partly fused to form a large bi-lobed extremity, m–m'. When this extremity had nearly touched the paramecium, it sent

out a small secondary pseudopod, a, beneath the prey, and b anterior to it (fig. 10). When the pseudopods a and b came in contact with the detritus, they moved apart and became much stouter (fig. 11). In the meantime a third pseudopod, c, appeared projecting from between a and b over the dorsal side of the paramecium, while a pocket was formed within the body proper of the ameba at the bases of these three pseudopods. The paramecium first jumped to position 2, figure 11. The excited paramecium next backed into the pocket of the body proper, 3, and a, b, and c closed in and surrounded it completely.

Usually ameba reacts to a free-swimming Euglena viridis by sending out pseudopods that widely embrace it. Sometimes, however, the embracing pseudopods close in upon the Euglena to hold it in a tight grip behind the position of the gullet, and this though the flagellum be quite active. On March 17, 1919, we saw a Euglena caught in this manner at its anterior end. The projecting part of the flagellate's body was passive, but the flagellum was very actively lashing within the enclosed bay. All movement for the time being had ceased in the gripping pseudopods. This observation had lasted for but a minute more or less when a large Paramecium, coming up at right angles to the Euglena, collided with it at the point indicated by the arrow in figure 12, and dragged the Euglena free from the ameba's grip. This was apparently the first step in the process of changing the second type of reaction into the first type. Mr. C. O. Dean, a student in this laboratory, observed an ameba that had thus gripped a Euglena viridis and thereby cut off its chance of escape. After the ameba had thus laid hold of the Euglena, its "ectoplasm flowed out around the Euglena" on all sides and so close to the wall of the Euglena that there was no water present between "the surfaces of the two organisms." This is not comparable to the food-taking by means of invagination as Prenard ('05) and Grosse-Allermann ('10) have described for Ameba terricola.

In 1900 the senior author observed a relatively small ameba ingest a relatively large Paramecium caudatum. In this case the ciliate was surrounded by pseudopods that were sent out

about it, but not touching it, about as Blochmann ('94) and Mast and Root ('16) indicate to be the usual method of ingesting paramecium. The latter authors saw some very interesting exceptions to this method of swallowing paramecium. We, too, have observed departures from this type of reaction. On May 2, 1919, we had a hanging drop in which there had been many Colpidia, but which were now dying off. · The dead ones, though frequently encountered by the ameba, were not in any case ingested. The living Colpidia were frequently accepted in wide embraces. The paramecia in this hanging drop were peculiar in that they were wider than normal ones and rather sluggish. Then, too, their bodies were so pliable that an ameba's pseudopod, advancing against the dorsal side of one of them, would indent it. Moreover, when the paramecia were crowded between two amebas, they became greatly flattened and even in some instances bent upon themselves at right angles. The cilia and contractile vacuoles of these peculiar paramecia were active. The amebas attacked these relatively inactive paramecia over and over; but in each instance their attack was peculiar in that they attempted to surround these ciliates closely or intimately. Because of this unusual method of attempting to capture the paramecia they caught none, for after two-thirds or less of the length of the paramecium's body had become involved in the embrace of the ameba, the paramecium would slowly glide out and remain by the side of the ameba until it would again be partially enclosed in a second embrace, when it would move out of the enclosing arms of its would-be captor. The conduct of the amebas toward these unusual paramecia is itself peculiar and exceptional. Here for some reason the ciliary disturbance of the water by the paramecia has not resulted in stimulating the amebas in such manner that they sent out about the prey remote encircling pseudopods.

A further departure from the usual method of ameba in capturing paramecium was observed March 19, 1919. This ameba was first seen at 10:10 A.M. It was then perfectly quiet, spending all of its available energy upon the partly constricted paramecium. The ameba showed no cytoplasmic movement (fig.

13).   At 10:15 A.M. the paramecium was further constricted and
the ameba quiet (fig. 14).   At 10:25 A.M. the cytoplasmic isth-
mus of the paramecium's body was stretched and the ameba dis-
played a little movement along pseudopod c and threw out pseu-
dopods a and b about the projecting portion of the paramecium,
the cilia of which were quite active (fig. 15).   Pseudopods a and
b were soon withdrawn.   At 10:35 A.M. the constricting and
stretching of the isthmus of cytoplasm were increased and the
isthmus was flexed (fig. 16).   By 10:43 A.M. the flexing of the
enclosed cytoplasm had become very conspicuous (fig. 17).   Two
minutes later a pseudopod, d, was sent out along one side of the
projecting lobe of the paramecium's body, the cilia of which were
yet quite active.   This secondary pseudopod was at once with-
drawn, while the paramecium was further stretched and bent.
At this phase of the reaction a Cyclidium darted into the field
and lay near the free end of a large 'anterior' pseudopod.   The
ameba reacted to this animal at once by sending out pseudopods
e and f and capturing the smaller ciliate (fig. 18).   The para-
mecium was now released by the ameba as it ingested the Cy-
clidium.   The constricted, elongated portion of the mutilated
paramecium shortened greatly and the large ciliate swam off
under its 'own steam,' having a contour about like the outline
given in figure 19.   No trace of cilia could be seen on the part
of the paramecium's body that had been ingested by the ameba.
    An ameba may ingest food at different parts of its body syn-
chronously.   We have observed one ingesting five Chilomonases
at one time and at five different regions of its body.   Moreover,
the two types of food reactions may be carried on simultaneously.
On January 28, 1919, while an ameba was ingesting a quiet fila-
ment of Oscillatoria, a Chilomonas came to lie beneath the fila-
ment at a position indicated in figure 20.   The Chilomonas was
lying beneath the plane in which the filament of Oscillatoria lay.
The ameba advanced about the plant until pseudopod b was
formed.   This pseudopod then sent out an encircling wall of
cytoplasm about the Chilomonas and then overarched it with
an ectoplasmic film.   The space within which the Chilomonas
was thus taken was next divided into a larger and a smaller

vacuole, the prey being in the smaller vacuole. The Chilomonas was not disturbed until it was thus enclosed within the smaller vacuole. The filament of Oscillatoria was further ingested, but it was finally rejected. Thus, while a reaction to a non-motile object was being carried on, the ameba completed a food reaction of the second type, in capturing a passive motile object.

Chilomonases have been seen to swim in beneath unattached regions of amebas' bodies. In such cases, when the amebas react positively to the flagellates, a curtain of cytoplasm is dropped down around the prey, the lips of which turn in beneath the food body and fuse without disturbing the Chilomonas.

Perhaps the most interesting reaction we have seen was that of an ameba reacting to a Chilomonas that had come to lie against the tip of a pseudopod (fig. 21, 1). The ameba sent out two pseudopods in response to the stimulus. The smaller pseudopod arose from the side of the parent pseudopod and a little behind its end, while the larger secondary pseudopod came out quite a distance behind the tip of the parent one. The interesting feature of this reaction is the fact that the parts reacting to the source of stimulation are parts least stimulated; indeed, the greater reaction was displayed by the least stimulated part. The quiet Chilomonas could stimulate the parent pseudopod in two ways: either chemically by means of its metabolic by-products, or physically by means of slight vortices that the play of its flagella may set up. In either case the end and not the sides of the parent pseudopod would be most affected by these stimuli. Moreover, we have studied the types of vortices set up in the water by quiet Chilomonases. This study showed that in all cases the strength of the currents thus set up was greatest at the anterior end of the Chilomonas. Finally, as the two secondary pseudopods were coming out by the sides of the Chilomonas, a second Chilomonas came to lie at position 2, figure 21, and thus double. or at least increase, the sources of stimulation; but this did not modify the conduct of the two secondary pseudopods. These facts indicate that the ameba's reaction is a qualitative and not a quantitative one.

DISCUSSION

It has been the tendency of recent work on the ameba to reduce the conduct of the ameba to simple terms. For example, Loeb ('05) says—"As a criterion for 'living matter' we might use the irritability or spontaneity. But as the 'spontaneity' of living matter is in its simplest form (in Amoebae) apparently not different from the physical phenomenon of spreading, for this criterion the limits of divisibility of living matter coincide with the limits of purely physical phenomena" (p. 321). McClendon ('09) tries to explain food-getting of ameba in the following manner: "Chemical and physical influences of the medium cause a hardening and shrinkage (by loss of water) of the ectosarc (Rhumbler's 'Geletinisrungsdruck'). Chemical processes within prevent this hardening from extending to the endosarc, and dissolve portions of the ectosarc that are displaced inward. The medium affects different portions of the surface to different degrees, causing regional differences in degree of hardening and shrinking, thus producing amoeboid movements. A food body being protoplasmic and therefore similar to the substance of the Amoeba might, in lying near an Amoeba, protect it from outside influences. The protected region would become more fluid, and shrinkage of other regions of the surface would press it out toward the food until it touched it. The food would be pushed along and sometimes rolled over and would rub on the surface of the pseudopod producing mechanical stimuli of sufficient frequency to cause a local shrinkage of the ectosarc. This stimulus would spread through the protoplasm but being very weak and rapidly growing weaker would cause the contraction of only a small area. Beyond the contracted area the protoplasm would continue moving toward the food and surround it from the sides" (pp. 268–269). But our observations indicate that the movement of an ameba about a food particle has little in common with "the physical phenomenon of spreading" and demands more than surface phenomena for their explanation of the food reactions of Ameba proteus. Of very recent date the students of the ameba have quite given up the idea that the

phenomena of spreading and surface tension are adequate for the explanation of the movement and food getting of this rhizopod. Hyman ('17) says "I am in favor of Clowes's" (16' *a*, '16 *b*) "interpretation, already referred to, that in passing from the surface of the protoplasm to the interior, a reversal of phase occurs, the colloidal material forming the outer phase, or disperse medium, in the surface layers, while in the interior it forms the disperse phase and water containing a variety of materials in solution and suspension, is the disperse medium" (p. 87).

It appears to us that even colloidal phenomena cannot be called upon to explain the phenomena involved in the food reactions of this ameba, because of their qualitative character. The qualitative nature of these phenomena becomes apparent when we compare the reactions of ameba to various quiet paramecia. In these reactions there appears a marked disparity between their variability and the degree of variability of the stimuli arising from the quiet animals that are about to be captured. An ameba may react to a quiet paramecium in three ways: 1) by forming a pocket within its own body within which the ciliate will be driven (figs. 9, 10, 11); 2) by sending encircling pseudopods about the prey and then roofing over and flooring the enclosed space with ectoplasm before disturbing the prey, and, 3) by closing in upon the paramecium with the advancing tips of two pseudopods until the prey is held fast in a grip of the pseudopods' ends. After the paramecium is thus caught, it is very tightly closed in upon and constricted (figs. 13 to 18). Paramecia concerned in 2 and 3 should not in a highly variable manner stimulate the ameba. It might be held that the feeding vortex of a paramecium lying with its ventral side directed toward a mass of detritus, as in 1, is of a different character from that of a paramecium lying free in the open. Then, too, the disturbance of the water by paramecium that had been caught at its girdle would be changed as soon as the vortex became divided by the advancing tips of the pseudopods and thus set up a new type of stimulation. There is weight to these possible objections that may be raised against the idea that the ameba's reactions toward paramecia are qualitative.

But in the case of the variability of the ameba's conduct toward Euglena there is less weight to these criticisms. Frequently Euglenas are taken into wide vacuoles while their flagella are lashing vigorously. When a Euglena is caught by the advancing ends of an ameba's pseudopods, as shown in figure 12, there is no reduction in the intensity of the stimulation, for the flagellum is quite as active as it was before the body of the Euglena was laid hold of. In this case the water in the forming vacuole is, if anything, more greatly disturbed than when a vacuole is forming about a Euglena that has not thus been grasped. Despite this lashing of the flagellum within the vacuole that had been formed, in the beginning, with reference to a Euglena free to move out of its embrace, the type of reaction was changed as soon as the body of the flagellate was held in the ameba's grip. This sort of modification of the ameba's conduct gives it a qualitative character rather than a quantitative one.

Finally the conduct of ameba toward Chilomonas paramecium indicates the qualitative character of its food reactions.

Chilomonas is captured only as it lies apparently quiet. This little saprophytic flagellate is a very active creature. Kent ('80) says this "animalcule rushes to and fro, though with the anterior end foremost, at a speed too rapid almost for the eye to follow, while at the next moment it comes as it were abruptly to anchor, with its body perfectly quiescent and one flagellum adherent to the glass slide or covering glass, while the other maintains a vibratory motion" (p. 425). By placing these animals in a mixture of India ink and water or by studying them in water containing many non-motile bacteria, we were able to determine the extent to which these apparently quiet Chilomonases disturbed the surrounding water. By this method, together with checks or controls of specimens in aquarium water, we were able to determine that but a very few anchor themselves as Kent describes. The smaller or ventral flagellum in this case lashes the water slowly in such manner that a vortex of water arises beyond the anterior tip of the Chilomonas, for about the length of its body, and this is drawn down over the gullet; from here it passes, at right angles to the axis of the body, to the dorsal side.

This current gains in velocity as it approaches the gullet; its velocity is greatly checked at the gullet, and by the time that it has passed the width of the body beyond the dorsal side of the Chilomonas, it has become quiet. The apparently quiet Chilomonas in this condition, therefore, disturbs the water within a very restricted area. The great majority of quiet Chilomonases are anchored by both flagella. These display a vibratory movement of short amplitude. But even in these cases we find the water is not remotely disturbed by the vibrations of the anchored Chilomonas. Not even the smallest suspended particles in the water were disturbed if they lay a body's length away from the vibrating animal. There is no other variation in the manner in which these animals disturb the surrounding water. The effect of Chilomonas upon the surrounding water seems, therefore, to be rather restricted (at no time passing beyond a body's length from the margin of the animal) and constant.

The great variability of the reactions of ameba to the stimuli arising from this rather restricted and constant disturbance of the water stands in sharp contrast to the constancy of the source of its stimulation. Kepner and Taliaferro ('13) showed that the reactions of Ameba proteus toward Chilomonas are qualitative ones. Our own observations on ameba feeding upon Chilomonas indicate that these reactions are qualitative. For example, in figure 5 we have represented a situation in which the ameba's pseudopods are traveling about the Chilomonas on all sides at a distance of about the length of the latter's body. It would appear, therefore, that in this case the ameba is following the limits of the waves that radiate from the vibrating animalcule. Many other examples might be given of amebas' making a much wider embrace of the Chilomonas, but there are some observations to be made that are more conspicuous in their contrast to the reaction recorded in figure 5. In the reaction shown in figure 21 there is no relation between the extent of the water's disturbance by the Chilomonas and the ameba's mode of capturing its prey. For here the regions of the ameba's body that reacted by sending out secondary pseudopods lay down beneath the tip of the broad parent pseudopod, so that these

regions, if not completely protected from the waves that radiated from the Chilomonas, were much less stimulated than was the broad end of the parent pseudopod; in addition to this, the smaller of these secondary pseudopods lay nearer the Chilomonas. This should have been the larger had the reaction been a quantitative one. Moreover, this reaction presents another interesting phase, for after it had well set in, a second Chilomonas entered the bay between the advancing secondary pseudopods. The amplitude of the water's agitation must now have been relatively greatly increased and yet the conduct of the two small pseudopods was not altered and they finally converged, though the stimulus had been increased. Here the qualitative character of the reaction is displayed in a manner exactly the opposite of that in which Kepner and Edwards ('17) saw a Pelomyxa act in a qualitative manner toward Paramecium, where, "though the stimulus was weakened the pseudopods continued to diverge as they grew" (p. 394) about the remaining paramecium of the three that were present at the inception of the reaction. Because of the qualitative character of the ameba's food reactions, it appears to us that these reactions toward an animal that presents the possibility of escape are modified with reference to meeting that contingency. The ameba's reactions differ, therefore, from chemical reactions in that they are made in the interests of the ameba and may be suspended or even reversed when its own interests demand.

SUMMARY

1. There are two general types of reaction to food: a) when no contingency of escape is presented by the prey, the ameba tightly surrounds the food; b) when such contingency is presented, a wide embrace is made and the prey is disturbed only when retreat is cut off.

2. These two types of food reaction are not fixed, but vary greatly.

3. In reacting to an object that usually moves in a horizontal plane, the ameba surrounds the prey in this plane first and next cuts off its vertical paths of escape.

4. A reaction is usually brought about through the coopera-
tion of both ectoplasm and endoplasm, though the ectoplasm
alone may carry out a reaction of the second type.

5. Both the ectoplasm and the endoplasm are highly contrac-
tile when conditions demand it.

6. The cutting of an animal like paramecium into two is pri-
marily a physical and not a chemical process—digestion setting
in after the prey has been defragmented.

7. The process of ingesting food is a reversible one. Food
half, almost, or wholly ingested may be egested.

8. An ameba's reactions differ from physical and chemical
phenomena in that they are qualitative rather than quantitative,
and are made in the interests of the acting organism.

LITERATURE CITED

BLOCHMANN, F. 1894 Kleine Mitteilungen über Protozoa. Biol. Centralb.,
        Bd. 14.
CLOWES, G. H. A. 1916 a Protoplasmic equilibrium. J. of Phys. Chem., vol. 20.
        1916 b Antagonistic electrolyte effects in physical and biological sys-
        tems. Science, N. S., vol. 43.
GROSSE-ALLERMANN, WILHELM 1910 Arch. Protistenkunde, Bd. 17.
HYMAN, LIBBIE H. 1917 Metabolic gradients in ameba and their relation to
        the mechanism of ameboid movement. Jour. Exp. Zoöl., vol. 24.
JENNINGS, H. S. 1904 Behavior of the lower organisms. New York.
KENT, W. SAVILLE 1880 A Manual of the Infusoria. London.
KEPNER, WM. A., AND EDWARDS, J. GRAHAM 1917 Food reactions of Pelomyxa
        carolinensis Wilson. Jour. Exp. Zoöl., vol. 24.
KEPNER, WM. A., AND TALIAFERRO, W. H. 1913 Reactions of Ameba proteus
        to food. Biol. Bull., vol. 24.
LEIDY, JOSEPH 1879 Fresh-water Rhizopoda of United States. Washington,
        D. C.
LOEB, JAQUES 1905 Studies in general physiology. Univ. of Chicago Press.
McCLENDON, J. F. 1909 Protozoan studies. Jour. Exp. Zoöl., vol. 6.
MAST, S. O., AND ROOT, F. M. 1916 Observations on ameba feeding on rotifers,
        nematodes and ciliates, and their bearing on the surface-tension the-
        ory. Jour. Exp. Zoöl., vol. 21.
PRENARD, E. 1905 Fauna Rhizop. du Bassin du Leman, Généve, Kundig 1902
        Arch. Protistenkunde, Bd. 6.

PLATES

1   First position of specimen with two small fragments of Oscillatoria within its body and in the act of ingesting a second filament of Oscillatoria.   × 100.

2   Second position after the ameba had been turned through 180 degrees by pushing against the partly ingested algal filament, a.   The ameba was given time to fix itself to the substratum in this position, when the Oscillatoria filament was a second time pushed upon.   This time the body of the ameba was not torn from the substratum and turned, but the tip of the ameba bent as the filament was pushed to position b.   × 100.

3   A very long, quiet Oscillatoria filament was being ingested when the projecting part of the filament was bent about the ameba to about the position shown in contour b.   When bent to this extent, the filament would slip from the glass rod and spring back as a straight rod.   The filament was thus bent and released twenty times before the ameba released its hold on the plant.   × 100.
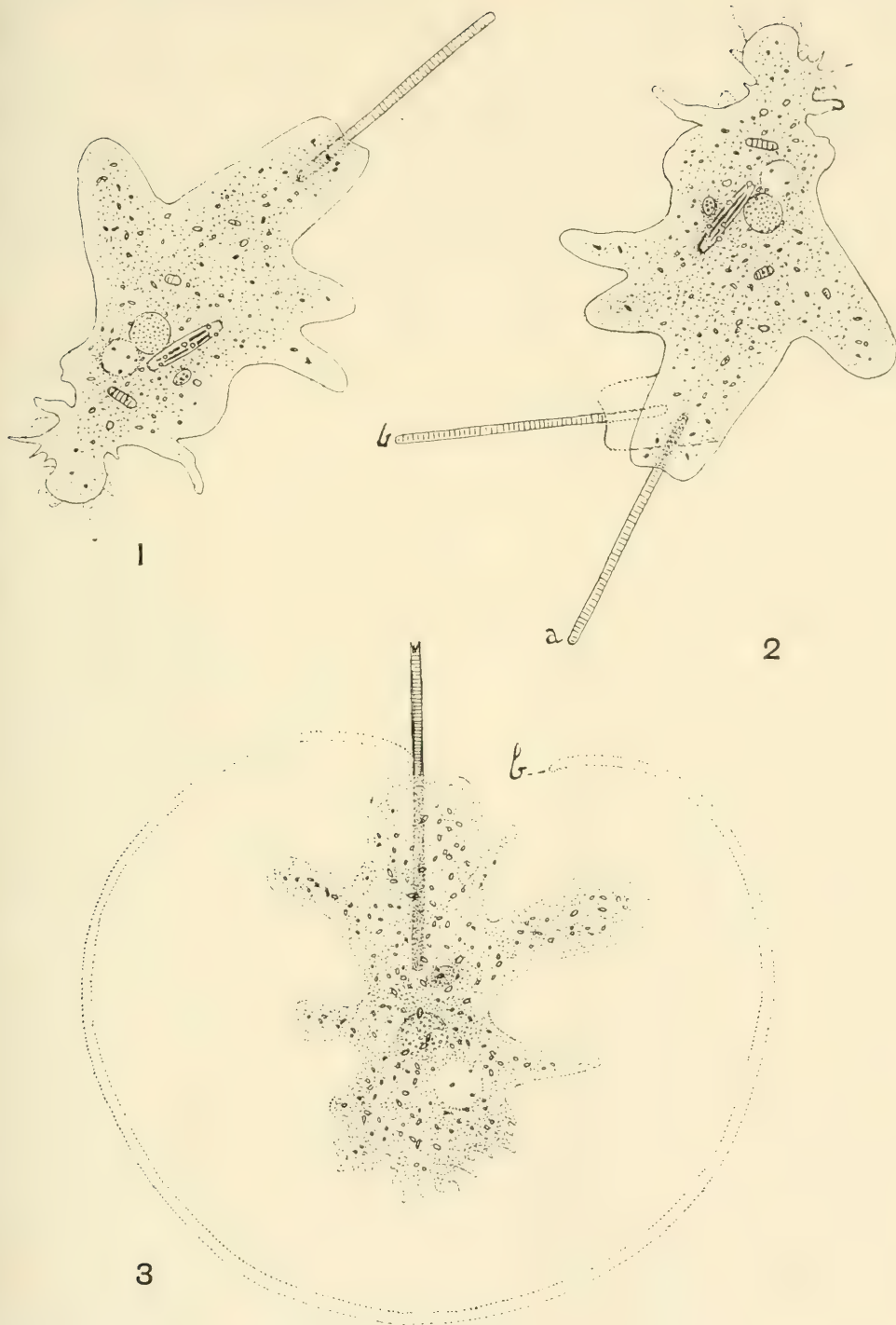
PLATE 2

4 Specimen reacting to a non-motile bacterial glea by constricting it with the pseudopods a and b. The larger lobe of the glea was ingested and broken up and the fragments delivered to small food vacuoles within the endoplasm of the ameba. $\times$ 200.

5 Specimen with fragments of Oscillatoria (O) in various stages of digestion (as shown by color) within the endoplasm. The ameba is cutting off the retreat of a quiet Chilomonas by means of the advancing pseudopods A and B. $\times$ 100.

6 An ameba against the side of which a motile mass of bacteria (A) was playing. The animal did not react to this stimulation. In reacting to some Chilomonases, that lay at the margin of a hanging drop in water that was shallow, the ameba sent out ectoplasmic pseudopods, a and a'. When a had grown to contour b an ectoplasmic wall was thrown over the top of the flagellates. These animals were thus caught in an ectoplasmic enclosure instead of one that was formed of both ectoplasm and endoplasm. $\times$ 200.

7 As the ameba was sending pseudopods a–b, and a' about two Chilomonases, a third Chilomonas came to lie along the outer margin of a'. In reacting to this third flagellate pseudopods c and c' were thrown out. $\times$ 200.

8 While pseudopods a and b were advancing along each side of a Chilomonas, they collided at the same time with a solid body. The growth of pseudopod b was now inhibited, while a advanced to contours c and d and finally surrounded completely the prey. $\times$ 200.

9, 10, 11   *y*, margin of a mass of detritus by which a paramecium was lying. 9, shows ameba advancing toward paramecium; 10, ameba sending pseudopods *a* and *b* forward until they made contact with detritus, after which they were moved apart and enlarged.   In the meantime a pocket was formed within the body of the ameba into which the paramecium moved as it moved to positions *2* and *3*.   × 200.

12   A ciliate being captured by pseudopods *a, b, c, d,* while a Euglena has been grasped as it was retreating from a forming food vacuole.   × 200.
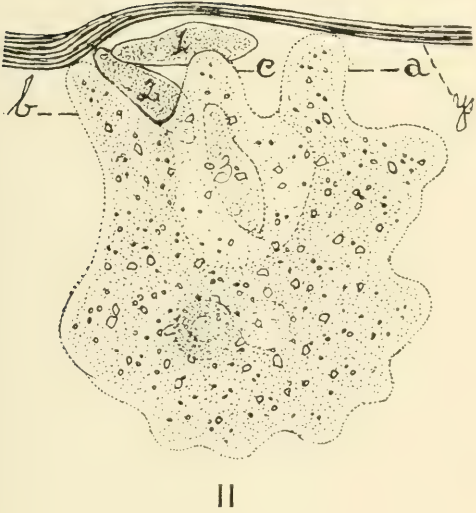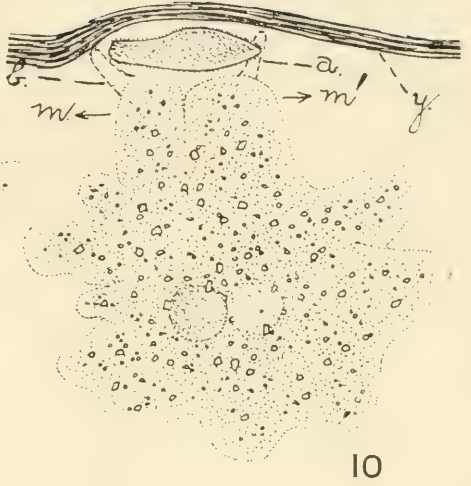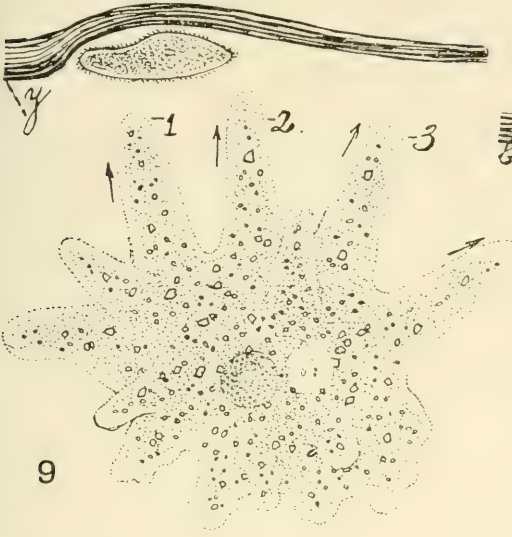
13, 14, 15   Figures show phases in the process of an ameba tearing a paramecium into pieces.   × 200.

13

15

14

PLATE 5

16, 17, 18   Show process of constricting and stretching the paramecium continued. e and f, pseudopods advancing about a ciliate.   As this small ciliate was being captured, the paramecium was released by the ameba. $\times$ 200.

19   Shows the shape the living paramecium assumed after it had been released by the ameba and swam away. $\times$ 200.

16

17

18

19
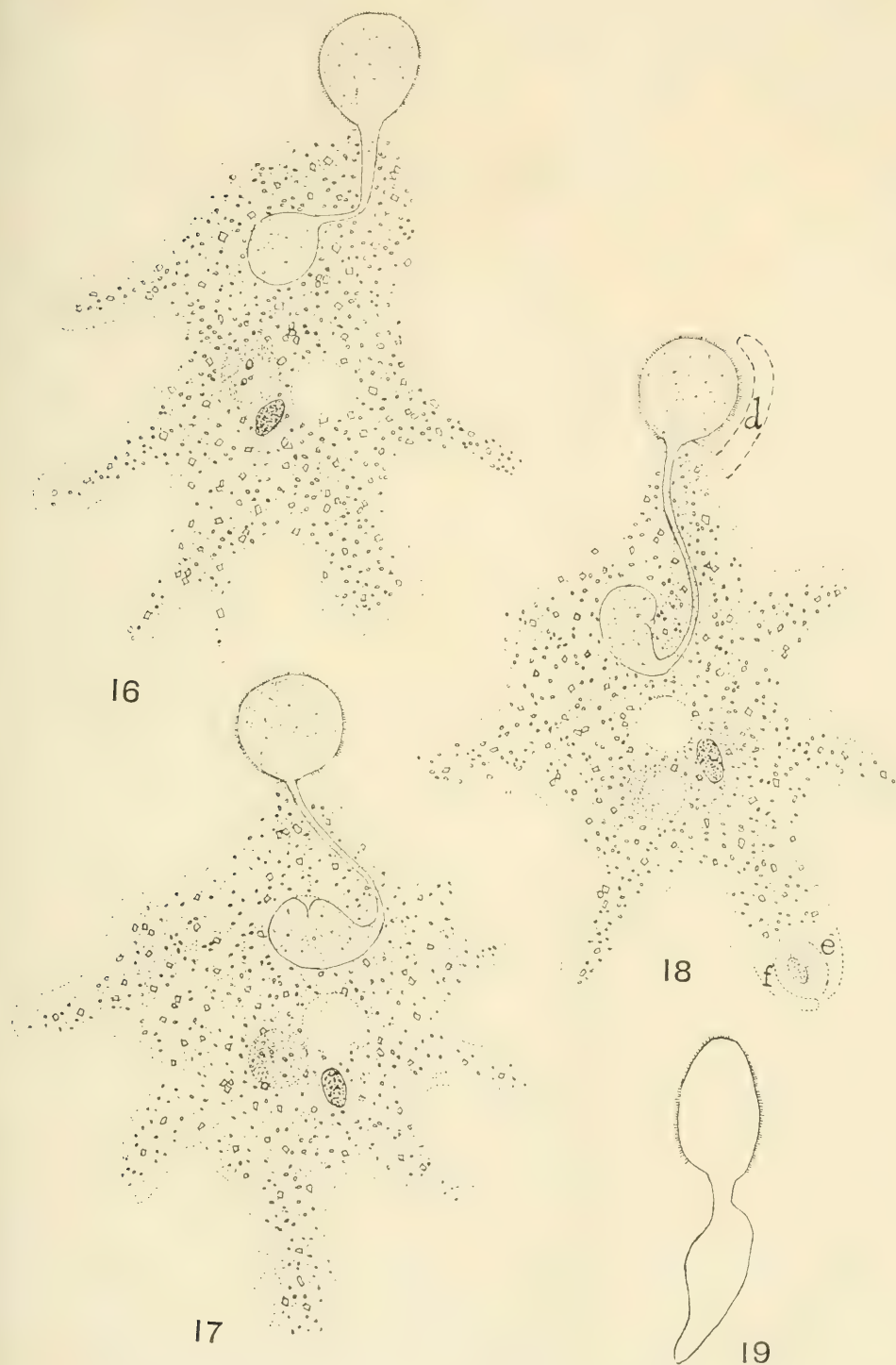
20 As the ameba was advancing about an Oscillatoria filament, a Chilomonas came to lie to the side of and beneath the alga. When the ameba had sent out the large pseudopod, *b*, it sent down beneath the alga a part of its body which enclosed the Chilomonas within a large vacuole before the prey was disturbed. Both types of food reactions were here carried on by the ameba synchronously. × 200.

21 An ameba capturing two Chilomonases which lay off the end of the parent pseudopod. × 200.

20
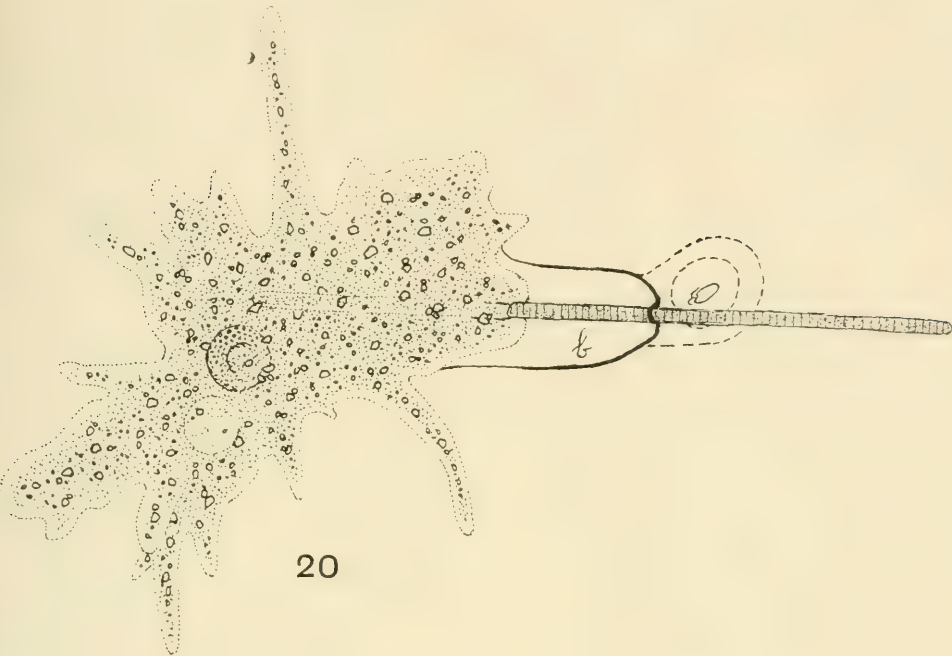
21

Resumen por el autor, Alfred O. Gross,
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    La alimentación y el sentido químico en Nereis virens Sars.

    Nereis virens no es un gusano carnívoro, como han creído
varios autores. Se alimenta principalmente de plantas que
crecen en las proximidades de los agujeros que habita, o de frag-
mentos de plantas transportados cerca del gusano por la marea.
El sentido del gusto o sentido químico no juega en Nereis una
parte muy activa en el hallazgo o la selección del alimento.
Nereis es fuertemente quimotrópico negativamente hacia los
ácidos, hidróxidos y sales. Es estimulado con mayor intensidad
por el cloruro potásico que por el cloruro sódico, al contrario de
lo que sucede en el caso de la lombríz de tierra. Esta diferencia
está relacionada con el tanto por ciento elevado del cloruro
sódico en el agua de mar, a la cual está adaptado Nereis.
    El tegumento general de Nereis es sensitivo a la acción de la
estimulación química, pero existe una localización o concentra-
ción del sentido químico en los palpos y tentáculos, cuya con-
dición está relacionada con la rica inervación de estos apéndices
y la relación de sus nervios con el cerebro. Aunque existe una
tendencia hacia la localización del sentido químico, este animal
no posee receptores especializados para recibir los estímulos
químicos. La localización del sentido del gusto en los palpos y
tentáculos debe explicarse mediante la existencia de alguna
cualidad específica diferenciada del protoplasma de estos
apéndices.

THE FEEDING HABITS AND CHEMICAL SENSE OF NEREIS VIRENS, SARS

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Nereis virens is a very common marine worm distributed along the Atlantic coast from Virginia northward to the Arctic regions. On the Pacific coast of America it is less common, but there are records of its occurrence from California northward to Puget Sound, Washington. In all favorable places of its range it occurs under stones or in burrows in the sand and mud of the intertidal areas.

Nereis is a very favorable animal for use in experimental work because of its abundance and the ease with which it may be kept alive in the laboratory for long periods of time. Since it is commonly used in the zoological laboratories as a type for dissection, a study of its habits seems desirable.

The experimental work on the chemical sense was conducted at the Marine Biological Laboratory, Woods Hole, Massachusetts. I wish to express my gratitude to Prof. G. H. Parker who suggested the problem and who has given me helpful criticism.

The fishermen and clam diggers along the New England coast believe that Nereis is dependent on the clam for its existence, hence the common name, 'clam worm.' Situations favorable for the clam are also attractive to Nereis, and as many of the worms find their way into the interior of dead snail shells or into the mud and sand between the two valves of the dead clams, the layman concludes that living molluscs are preyed upon and killed by Nereis. Zoologists, if they have any conception at all of the feeding habits of Nereis, believe it to be a carnivorous worm, whose powerful jaws are for the purpose of capturing and tearing other marine animals. In all probability,

this idea has arisen from certain published statements such as the following made by Prof. A. E. Verrill on page 318 of his report upon the invertebrate animals of Vineyard Sound. "It is a very active and voracious worm, and has a large, retractile proboscis, armed with two strong, black, hook-like jaws at the end, and many smaller teeth on the sides. It feeds on other worms and various kinds of marine animals. It captures its prey by suddenly thrusting out its proboscis and seizing hold with the two terminal jaws; then withdrawing the proboscis, the food is torn and masticated at leisure, the proboscis, when withdrawn, acting somewhat like a gizzard." This statement apparently was taken at its face value, and we find it copied into the various text-books and natural histories, of which the following taken from the Standard Natural History (vol. 1, p. 229) is one of many examples: "It is a very active and voracious worm terrible to smaller animals upon which it preys capturing them by its large proboscis which it suddenly thrusts out seizing its victim with the two large jaws which arm the tip of its efficient weapon of attack," etc. Verrill's statement has also misled investigators who have taken it for granted that the food of Nereis is animal.

Prof. S. S. Maxwell, in his paper on the physiology of the brain of annelids, quotes Verrill, and later, on page 283, he describes the normal feeding reactions of Nereis virens as follows: "Wenn man ein Stück Fütter, z. B., ein kleines Stück von einem Wurm, auf eine Nadel spiesst und vorsichtig einem normalen Wurm reicht, kann man den Fressvorgang leicht sehen. Wenn man das Futter den Spitzen der vorgestreckten Fühler nähert, kommt der Wurm gewöhnlich ruhig näher. Dann zieht er den Kopf ebenso ruhig ein wenig zurück, legt die Fühler an den Körper und öffnet den Rachen, um die Nahrung zu fassen."

It was with the above conception that Nereis virens was a carnivorous worm, that the author began experiments on the sense of taste. A voracious worm whose food is other animals would be expected to have well-developed organs of taste. Various experiments were subsequently devised in an attempt to study the normal feeding reactions of the worms. Entire, as

well as extracts and ground-up messes of marine worms, crustaceans, molluscs, fish, etc., would not tempt even a semistarved individual to eat. Though Nereis never utilized the animal substances provided as food, it ate freely of the sea lettuce which had been introduced into the dishes to aerate the water. Thinking that possibly laboratory conditions had so altered the physiological conditions of the worms, that its feeding habits were abnormal, observations were made in the field. In no case was a Nereis seen to prey upon living animals, but many were observed to eat vegetable matter. To substantiate these observations examinations were made of the intestinal contents of a number of worms collected from various situations in several localities as shown in the following table:

TABLE 1

LOCALITY	NUMBER OF WORMS EXAMINED	CONTENTS OF THE ALIMENTARY TRACT GIVEN IN APPROXIMATE PERCENTAGES
Naushon Island, Woods Hole, Mass.	35	Eel-grass, 75 per cent Various algae, 15 per cent Sand, mud and miscellaneous material, 10 per cent
Juniper Point, Woods Hole, Mass.	10	Nemaleon (an alga), 75 per cent Sphacelaria (an alga), 15 per cent Sand, egg masses and miscellaneous material, 10 per cent
Eel Pond and Buzzards Bay, Mass.	38	Roots and blades of eel-grass, 95 per cent Sand, mud, sponges, Bryozoans and miscellaneous material, 5 per cent
Lynn Beach, Lynn, Mass.	12	Eel-grass, 55 per cent Algae, 25 per cent Sand and mud, 15 per cent Miscellaneous material, 5 per cent
New Meadows River (salt), Brunswick, Maine	20	Rock weed, 95 per cent Mud and miscellaneous material, 5 per cent

The results of this examination show conclusively that Nereis virens is not an animal feeder, but is primarily a vegetarian.

Furthermore, this worm is able to adapt itself to a large range of plants for food and utilizes that which is abundant and most convenient to its burrows. The jaws and proboscis are used extensively in excavating burrows, but, as compared with the earthworm, a relatively small amount of sand and mud is ingested by Nereis. The animal materials, such as bryozoans, sponges, and egg masses, found in the intestine were originally attached to the plants eaten by Nereis and were probably an accidental element of the food. The worms seemed to exhibit no preference for eel-grass covered with bryozoans and egg masses, nor did they shun such material when they chanced to come upon it.

When a number of Nereis are crowded into a small dish they may, especially if mechanically or chemically stimulated, violently thrust out the proboscis, extend the jaws, and bite the body of a fellow worm so severely as to sever it in two parts. I have seen a worm bite its own body in two when placed under pressure or treated with a strong acid or alkali. In such cases it may incidently take into its proboscis some of the flesh which is grasped. Very often, when ejecting extracts of animal juices from a pipette toward the head of the worm, it would thrust out its proboscis, just as it did when treated with an acid or alkali. These thrusts I soon learned were not attempts at securing food, but were acts of self-defense and, it is very probable they often serve the worms as an effective protection against enemies as large or much larger than itself. The feeding response is a much more deliberate act. Is it not possible that an observation, such as noted above, and the fact that other species of Nereis have been reported as animal feeders, may be primarily responsible for Professor Verrill's erroneous statement, a record which has been copied so many times without any attempt at verification?

The jaws, though not used in capturing animal prey, are employed in tearing out bits of the plants used as food. In the intestines of some of the larger individuals it was not uncommon to find pieces of eel-grass or other vegetation 1 to 2 cm. in length.

A number of experiments were made with the natural food in an effort to localize the sense of taste, but the worms showed no

consistent responses to food of any kind. They fed freely upon sea lettuce and other plants placed in the aquaria, but the finding of it was more or less accidental. Food hidden in sand, placed in cheese-cloth bags, or otherwise concealed was, as far as could be determined, never detected by the worms. Animals from which one or all of the pairs of cephalic appendages, such as the tentacular cirri, palps, and tentacles, were removed, fed and thrived as well as normal animals.

It is evident that the sense of taste, or chemical sense, of Nereis virens does not play an important rôle in locating and selecting food. It is conceivable, however, that a chemical sense may be developed which enables the worm to detect certain unfavorable environmental conditions of the water and mud in which it lives.

To test the chemotropism of Nereis, simple reagents, such as HCl, KOH, NaOH, KCl, NaCl, and NH$_4$Cl, were used. The worms were tested by the various methods used by Parker, Hurwitz, Shohl, Crozier, and Irwin on the earthworm. The fence method used by Shohl proved to be the most satisfactory for the experiments on Nereis. For this purpose a rectangular, shallow glass tray was divided into two compartments by a paraffin partition, a quarter of an inch wide. A notch about three-quarters of an inch long and reaching within a half inch of the bottom, was cut in the middle of the partition. The entire tray was covered over with a thin layer of paraffin, to prevent the liquids from wetting the walls. Sea-water was placed on one side of the fence and sea-water containing the stimulating substance on the other side. The worms were transferred from the individual dishes in which they were kept to the notch in the fence by means of two paraffin-covered wooden spatulas. The worms thus placed were free to crawl into the liquid toward which their anterior end was directed or to withdraw into that on the opposite side of the fence. Nereis was found to be very strongly negatively chemotrophic to all the reagents used. The reaction times of the worms, which increased inversely as the strength of the stimulating substances, were recorded by means of a stop-watch controlled by foot pressure.

When the worms were placed on the fence with sea-water on one side and ordinary tap-water or distilled water on the other, the worms quickly withdrew into the sea-water, indicating that the latter has a marked disturbing effect on Nereis. Because of this condition, the special substances used as stimuli were always added to the sea-water. It may not be safe, by this method, to make a comparison of the relative stimulating efficiency of one acid with another or with an alkali, hydroxide, or salt, because of the many substances in solution in sea-water which might affect the reagent. One can, however, make comparisons of the relative sensitiveness of the worms under different conditions. As long as there is a constant stimulating liquid in the mixture of a measured quantity of sea-water and a definite amount of the chemical, it makes no difference for this purpose what the resulting chemical combinations and mixtures may be.

For convenience of comparison the various reagents were made up in molecular solutions and these solutions were added in definite quantities, by means of a burette, to the sea-water in the following proportions.

TABLE 2

1 cc. mol. HCl	to 300	cc. sea-water
1 cc. mol. KOH	to 10	cc. sea-water
1 cc. mol. NaOH	to 10	cc. sea-water
1 cc. mol. KCl	to 10	cc. sea-water
1 cc. mol. NaH$_4$Cl	to 3	cc. sea-water
1 cc. mol. NaCl	to $\frac{1}{5}$	cc. sea-water

The worms exhibited a marked reaction when tested with mixtures of sea-water and molecular solutions in the proportions shown in the above table.

The reaction times of the worms when tested with these solutions were short, but not too short to be accurately measured by means of a stop-watch. Though these concentrations of salts produced only approximately similar reaction times, it is interesting to note that it required about fifty times as great a concentration of NaCl as KCl to produce an approximately similar reaction time on Nereis; whereas Parker and Metcalf found NaCl to be more stimulating than KCl to the dung earthworm, Allolobophora foetida. This striking difference is probably

correlated with the high percentage of NaCl and the low percentage of KCl in the sea-water to which Nereis is adapted. The author hopes to perform experiments on this interesting and important aspect of the problem which involves the relations of osmotic pressure and sense of taste to the stimulating substances used as well as the relative stimulating efficiency of the various reagents.

This paper involves only those experiments made on Nereis in an effort to determine whether the chemical sense is localized in certain cephalic appendages or in other parts of the worm. For a preliminary test twenty-four worms of a uniform size (10 to 12 cm. long) were numbered and placed in separate finger-bowls, each containing 20 to 25 cc. of sea-water and a small piece of sea lettuce. The latter aerated the water and provided food for the worm. Each individual of the whole series of Nereis was given one test in its turn with the HCl, then one test with the KOH, and so on until the entire set of readings for each of the two reagents was obtained. The order of the worms in the test was reversed each time a new series of readings was taken. All the experiments were made under conditions controlled for temperature and light, and, as far as possible, free from mechanical stimulation. After each individual test, the worm was rinsed in fresh sea-water before being returned to its bowl. The sea-water containing the stimulating substance, as well as the plain sea-water, was renewed after each set of readings, since a small amount of the liquid was carried from one side to the other by the worm.

After the reaction time of the worms had been determined, the tentacular cirri, palps, and tentacles of the first twelve of the twenty-four Nereis were removed, while the other twelve of the series were retained in a normal condition to be used as a control. The entire series was left undisturbed for a period of six days, a length of time more than sufficient for the wounds made by the operations to heal. Readings were then made as before to determine what effect, if any, the removal of the cephalic appendages had on the sensitiveness of the worms to chemical stimulation.

The average reaction time was very much lengthened in the case of the twelve individuals from which the appendages were removed, but it remained practically unchanged in the unoperated animals used as a control. After these determinations were made, the worms were put aside until the appendages were completely regenerated. With the regrowth of the appendages the sensitiveness of the worms to chemicals was restored, as evidenced by the reaction time which became about equal to that of the control animals and to that of the same worms before the operations were made. The results of this preliminary experiment indicate that certain of the appendages of the head region are more sensitive to chemical stimulation than the general integument of the worm. In order to determine whether the chemical sense is shared equally by all the appendages or more strongly developed in some than in others, the experiments were repeated, but with this difference, only one of the three pairs of cephalic appendages was removed from the worms of any one series. The anal cirri were likewise tested for their sensitiveness to chemical stimulation. The following tables contain the results of the tests made upon Nereis virens under the various conditions indicated. In each case the number of animals used, the number of readings made, and the mean of the reaction times with the probable error is given. It is deemed impracticable to publish the individual readings which, with the preliminary tests, involve more than 2000 determinations. For convenience in comparing the reaction times of the worms under two conditions, the difference of the means of the reaction times and the 'significance factor' are also given in the tables.

The significance factor involves a comparison of the means of the reaction times including their probable errors. The value of this factor is obtained by dividing the difference of the means of the reaction times of the normal and operated animals by the square root of the sums of the squares of the probable errors of these two reaction times. As an example, take the case of the palps in table 3 in which the worms were tested with KOH. The reaction time of the normal animals is 9.44 seconds with a probable error of 0.479, and the reaction time of the same set of

TABLE 3

Table containing results of the experiments on the reactions of Nereis virens to potassium hydroxide. Concentration, 1 cc. molecular KOH to 10 cc. of sea-water

	Number of animals	Number of readings	Mean of reaction times	Probable error of the mean of reaction times		Number of readings	Mean of reaction times	Probable error of the mean of reaction times	Difference of the means of reaction times	Factor of significance of data
Tentacular cirri, all organs normal......	5	25	8.26	0.526	Tentacular cirri removed.....	25	10.09	0.563	1.83	2
Control (normal).............	6	30	9.58	0.507	Control (normal).........	30	10.54	0.464	.96	1
Palps, all organs normal.....	5	25	9.44	0.479	Palps removed........	25	21.57	1.45	12.13	8
Control (normal).............	6	30	9.58	0.507	Control (normal).........	30	10.54	0.464	.96	1
Tentacles, all organs normal...	5	60	6.57	0.381	Tentacles removed.......	60	12.76	0.465	6.19	12
Control (normal).............	4	48	7.82	0.299	Control (normal).........	48	9.33	0.422	1.51	2
Anal cirri (normal)..........	5	60	8.31	0.403	Anal cirri removed.......	60	8.29	0.421	0.02	1—
Control (normal).............	4	48	6.32	0.348	Control (normal).........	48	7.82	0.481	1.5	2

TABLE 4

Table containing results of the experiments on the reactions of Nereis virens to hydrochloric acid. Concentration, 1 cc. molecular HCl to 300 cc. of sea-water

	Number of animals	Number of readings	Mean of reaction times	Probable error of the mean of reaction times		Number of readings	Mean of reaction times	Probable error of the mean of reaction times	Difference of the means of reaction times	Factor of significance of data
Tentacular cirri, all organs normal	5	25	1.52	0.081	Tentacular cirri removed	25	2.06	0.097	0.54	4
Control (normal)	6	30	1.85	0.116	Control (normal)	30	1.75	0.085	0.10	—
Palps, all organs normal	5	25	1.43	0.14	Palps removed	25	3.34	0.284	1.91	6
Control (normal)	6	30	1.85	0.116	Control (normal)	30	1.75	0.085	0.10	—
Tentacles, all organs normal	5	60	1.45	0.066	Tentacles removed	60	2.58	0.175	1.13	6
Control (normal)	4	48	1.93	0.081	Control (normal)	48	2.19	0.0998	0.26	2
Anal cirri, all organs normal	5	60	2.28	0.095	Anal cirri removed	60	2.32	0.087	0.04	1 —
Control (normal)	4	48	2.28	0.044	Control (normal)	48	2.49	0.108	0.21	1 —

TABLE 5

Table containing results of the experiments on the reactions of Nereis virens to ammonium chloride. Concentration, 1 cc. molecular NH₄Cl to 3 cc. of sea-water

	NUMBER OF ANIMALS	NUMBER OF READINGS	MEAN OF REACTION TIMES	PROBABLE ERROR OF THE MEAN OF REACTION TIMES		NUMBER OF READINGS	MEAN OF REACTION TIMES	PROBABLE ERROR OF THE MEAN OF REACTION TIMES	DIFFERENCE OF THE MEANS OF THE REACTION TIMES	FACTOR OF SIGNIFICANCE OF DATA
Tentacular cirri, all organs normal........	5	25	15.13	1.17	Tentacular cirri removed.....	25	14.52	0.86	0.61	1 —
Control (normal)............	6	30	15.19	1.03	Control (normal).........	30	16.92	0.66	1.73	1
Palps, all organs normal......	5	25	13.87	0.97	Palps removed............	25	29.71	1.87	15.84	7.5
Control (normal)............	6	30	15.19	1.03	Control (normal).........	30	16.92	0.66	1.73	1

animals with their palps removed is increased to 21.57 seconds with a probable error of 1.45. Substituting the values in the formula as above stated, we have

$$\frac{21.57 - 9.44}{\sqrt{(.479)^2 + (1.45)^2}} = \frac{12.13}{1.52} = 8-$$

A significance factor greater than about 3 signifies the results are to be considered of scientific value. No importance is to be attached to the difference in reaction times if the significance factor falls below 3. Furthermore, if this factor becomes greater than 3 in the two sets of readings of the control, then the results of the experiments become questionable, either because of lack of care in performing them or because certain factors, such as light, temperature, etc., were not properly kept under control. An examination of the tables at once reveals the fact that the palps and tentacles are so highly sensitive to chemical stimulation that their removal causes a marked change in the reaction times of the animals. The tentacular cirri, which together have a much greater exposed surface than the tentacles and palps combined, are sensitive to a much less degree; indeed, in only the HCl test was there a noticeable change in the reaction time when the eight tentacular cirri were removed. The significance factor in this case is only 4, so even here the difference in reaction time becomes of doubtful value. The anal cirri, though they are sensitive to chemical stimulation, are not sensitive to the degree that their removal causes a measurable change in the responsiveness of the worms.

DISCUSSION

The fact that the palps and tentacles are much more sensitive to chemical stimulation than the tentacular cirri becomes of more interest when the innervation of these appendages is considered. The palps and tentacles are supplied with well-developed nerves, which arise directly from the supra-oesophageal ganglion or brain, whereas the two pairs of tentacular cirri are innervated by nerves which have a very different origin. The

nerves of two pairs of tentacular cirri arise from the sub-oesoph-
ageal ganglion, and those of the others take their origin from the
circumoesophageal connectives. In the higher animals, the nerves
of special sense, such as sight, taste, etc., are directly connected
with the brain. It is reasonable to infer that in a highly organ-
ized worm like Nereis, we have the beginnings of a concentra-
tion of sense receptors into more or less limited regions which
have become secondarily but directly related to a centralized
brain. This localization of the chemical sense has not pro-
gressed to any great degree, for the whole general integument of
Nereis, though less sensitive than the palps and tentacles, is
open to chemical stimulation. The same is true with the light
sense. The general integument of Nereis is sensitive to light,
yet there is a tendency toward a localization of the light sense
in the presence of two pairs of relatively well-developed eyes.
These eyes are innervated by large nerves which connect directly
with the brain. The conditions of these sense organs in Nereis
are intermediate between those forms in which there is only the
general integumentary sense and the higher forms in which the
chemical sense is vested solely in special sense organs innervated
by cranial nerves.

Maxwell has attempted to show that the feeding responses,
that is responses due to chemicals or substances given off by
food, cease when the supra-oesophageal ganglion is removed.
Maxwell's statement is as follows: "Operirte Würmer beachten
dagegen angebotenes Futter gar nicht, es sei denn, dass man es
unsanft auf sie wirft und sie dadurch erschreckt. Sie kriechen
über Stücke Futters, die in ihrem Wege liegen, als ob es Steine
oder anderes lebloses Material wäre. Obschon ich diese Würmer
viele Wochen hindurch gehabt habe, ist es mir nicht in einem
einzigen Falle gelungen, sie zu füttern. Mit dem Verlust des
supraösophageschen Ganglions scheint das Thier die Fähigkeit
verloren zu haben die spezifischen Reaktionen auf die chemischen
Reize, die vom Futter ausgehen, zu zeigen."

Maxwell's experiments show that the removal of the supra-
oesophageal ganglion changes the responsiveness of the worms to
chemical stimulation—a result which is in direct line with what

I have found. When the above ganglion is removed, the animal
is less sensitive to chemicals, because that part of the chemical
sense which resides in the palps and tentacles is lost. The
nerves of the tentacular cirri were left intact, as evidenced by
the fact that the cirri were still responsive to mechanical stimula-
tion. Unfortunately, Maxwell's experiments are of less value
from the standpoint of their bearing on the sense of taste because
his observations, as before noted, are not of the feeding reactions
of Nereis. The responses he secured by holding a piece of worm
flesh in front of Nereis was merely the characteristic defensive
thrust, due to chemical stimulation or irritation. These
responses are, at best, very irregular and erratic and cannot be
used in careful comparative work. In the tests on the operated
worms Maxwell placed the stimulating substances, i.e., pieces
of worm flesh in the sea-water containing the Nereis. Under
such conditions it is difficult to detect and impossible to meas-
ure quantitatively the effect of chemical stimulation on the
worm. To such a liquid the worms soon became adapted and
not stimulated at all. That the operated worms exhibited no
feeding reactions under these conditions is perfectly obvious,
because even a normal Nereis does not feed upon flesh, with
which Maxwell tested the worms. I have found that worms
still respond to chemical stimulation after the brain is removed
if tested by the method previously described. This chemical
sense of the general integument evidently works through a
ganglionic reflex, that is, through the ganglia of the ventral
nerve cord.

In addition to the rich innervation of the palps and tentacles,
as shown by Retzius, there is an abundance of diffuse integu-
mentary sense organs to be found on these appendages. Lang-
don has shown these organs to be especially numerous on the
tentacles and on the tips of the palps. But since these organs
are also abundant on the tentacular, parapodial, and anal cirri,
their significance in connection with the sense of taste is at
least a doubtful one. From the standpoint of distribution,
the evidence is to the contrary, and I am inclined to believe
these integumentory sense organs, which are also abundant

in the earthworm, are purely tactile. Any evidence that the so-called 'spiral organs' are chemical receptors is also lacking. The localization of the sense of taste in the palps and tentacles must be explained by some differentiated specific quality of the protoplasm of these appendages. In Nereis there is a beginning of the localization of the sense of taste or chemical sense, but there have not as yet developed specialized receptors (taste buds) for the reception of chemical stimuli.

CONCLUSIONS

1. Nereis virens is not a carnivorous worm as stated by Verrill and others.

2. Nereis feeds chiefly upon plant life.

3. The sense of taste or chemical sense of Nereis plays a small part, if any, in locating or selecting food.

4. Nereis is strongly negatively chemotrophic to acids, hydroxides, and salts.

5. Nereis is stimulated much more by potassium chloride than by sodium chloride—a reverse of the conditions found in the earthworm. This difference is correlated with the high percentage of sodium chloride in the sea-water to which Nereis is adapted.

6. The general integument of Nereis is sensitive to chemical stimulation, but there is a localization or concentration of the chemical sense in the palps and tentacles—a condition correlated with the rich innervation of these appendages and the relation of their nerves to the brain.

7. Though there is a tendency for a localization of the chemical sense, there are no specialized receptors, taste buds, for receiving chemical stimuli in Nereis virens.

BIBLIOGRAPHY

CROZIER, W. J. 1916 Cell penetration by acids. Jour. Biol. Chem., vol. 24, pp. 255–279.

HURWITZ, S. H. 1910 The reactions of earthworms to acids. Proc. Am. Acad. Arts and Sciences, vol. 46, pp. 67–81.

IRWIN, M. 1918 The nature of sensory stimulation by salts. The Amer. Jour. of Physiol., vol. 17, pp. 265–277.

LANGDON, F. E. 1900 The sense-organs of Nereis virens Sars. Jour. Comp. Neur., vol. 10, pp. 1–78.

MAXWELL, S. S. 1897 Beiträge zur Gehirnphysiologie der Anneliden. Archiv für die gesammte Physiologie, Bd. 67, S. 263–297.

PARKER, G. H., AND METCALF, C. R. 1906 The reactions of earthworms to salts. Amer. Jour. Physiol., vol. 17, pp. 55–74.

RETZIUS, G. 1895 Zur Kenntniss des gehirnganglions und des sensiblen Nervensystems der Polychäten. Biol. Untersuchungen, Bd. 7, S. 6–11.

SHOHL, A. T. 1914 Reactions of earthworms to hydroxyl ions. Am. Jour. Physiol., vol. 34, pp. 384–404.

STANDARD NATURAL HISTORY 1885 Lower invertebrates, vol. 1, pp. 1–389. Boston, S. E. Cassino & Co.

VERRILL, A. E. 1873 Report on the invertebrate animals of Vineyard Sound and the adjacent waters. U. S. Comm. of Fish and Fisheries, part 1, pp. 295–778 (Nereis, p. 318).

Resumen por el autor, W. J. Crozier,
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Sobre la historia natural de Onchidium.

El presente trabajo contiene una descripción de las activi-
dades de Onchidium floridanum, miembro de un grupo de pul-
monados semi-marinos notables por sus rasgos estructurales de
naturaleza enigmática, y, como demuestra este trabajo, también
por sus costumbres no menos notables. A una discusión de
las reacciones sensoriales sigue un análisis comparativo del
heliotropismo y especialmente de la conducta de este animal al
buscar una habitación. El autor insiste especialmente sobre el
carácter no adaptativo del heliotropismo en Onchidium y sobre
la interpretación mecanística de los fenómenos que exhibe al
buscar un albergue.

ON THE NATURAL HISTORY OF ONCHIDIUM[1]

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CONTENTS

INTRODUCTION

Onchidium (Onchidella) floridanum Dall belongs to a group of naked pulmonates (Pelseneer, '01, p. 21) which, after the long discussion concerning the obscure morphology of their respiratory apparatus (cf. Joyeux-Laffuie, '82; Bergh, '95; v. Wissel, '98), have been chiefly remarkable for their littoral marine habitat, and, more conspicuously, for the eyes—of a structural type unique among gastropods (Semper, '77; Stantschinsky, '07; Hirasaka,[2] '12)—developed by some of them upon their

[1] Contributions from the Bermuda Biological Station for Research, no. 126.

[2] We are indebted to Mr. T. Minoura, of the zoölogical department, University of Chicago, for a knowledge of Hirasaka's paper, and for his kind translation of essential portions of its contents, as well as for his translation of Fujita's ('97) account of the respiration of the Japanese Onchidium.

dorsal surface. In connection with these strange eyes and their supposed functional significance (Semper, '81; Bretnall, '19) a few observations and some suppositions have from time to time been recorded with reference to the behavior of Onchidium; but knowledge of its life and habits, which we find to exhibit some perplexing features of curious interest, has been strangely meager; nor has proof been offered that the 'eyes' are indeed photo-sensitive (cf. Crozier and Arey, '19c).

At Bermuda, O. floridanum is quite generally distributed along the shores of the islands, commonly inhabiting protected locations where the intertidal shore zone is covered by a layer of sun-bleached algae, although it is also found in intertidal habitats of other kinds (Crozier and Arey, '19a). O. floridanum is an Antillean species closely resembling others of this genus found in the Pacific (Dall and Simpson, '01). Like most of its relatives, this species is strictly intertidal; some forms have been recorded as dwelling beneath low water.

Onchidium is a good laboratory animal. It can be maintained in small dishes with a little sea-water for a month or longer, even if starved. In one case several individuals were kept in small (50-cc.) bottles, tightly stoppered, and half full of sea-water, in which diatoms had been planted. They were alive, active, and of normal appearance at the end of six weeks. The mollusks were observed to creep above the water in the bottles at irregular intervals, later returning under water and feeding upon the plant growths there.

For experimental observations it is nevertheless desirable that freshly collected animals be employed, and this has been the rule in the work forming the basis of the following discussion. Our material came, in 1914–17, from Little Agar's Island, and in 1918 from the shore of Dyer Island, in each instance very close to the locations of the Biological Laboratory in these years. In addition, much of what we have learned concerning the behavior of Onchidium is the result of sun-baked vigils on the shore rocks in other parts of Bermuda. The work was initiated in 1914 by L. B. A., and has since then been extended by W. J. C. The observations here collected pretend to no

exhaustiveness; they do, however, throw valuable light upon some vexed questions, particularly in connection with the theory of adaptation. It is therefore important to remark that upon some of the peculiar features in this account of the behavior of Onchidium our respective observations, secured in an entirely independent manner, have been found in essential agreement at every point of overlapping. Elsewhere we have already commented on certain points in the behavior of Onchidium (Arey and Crozier, '18; Crozier and Arey, '19 a, 1919 c). It is our purpose here to bring together in a unifying way the results of our previous communications, presenting fully the evidence concerned and indicating its place in a more systematic account of the natural history of this animal.

OCCURRENCE; HOMING ACTIVITIES; COLORATION; REPUGNATORIAL GLANDS

If, during the period of low water, on a sunny day, one inspect the shore rocks at Bermuda laid bare by the tide, at a place protected from the dashing of the surf, one frequently has little difficulty in discovering numbers of small blue-black slug-like Onchidia, about 15 mm. long or smaller. They feed quietly upon the thin coating of felted algae or creep about in a manner which at first sight seems aimless. Favorable localities are found on the lee shores of smaller islands within the semi-enclosed sounds (Great Sound, Castle Harbor, but not in Harrington Sound, where there is practically no tidal rise and fall and where no Onchidia occur). The animals also live, however, in bays on the north and south shores of Bermuda, but not where they would be directly exposed to the ocean surf.

The body of the adult Onchidium when resting undisturbed is dome-like, oval in outline, at most 17 mm. long × 12 mm. broad × 6 mm. high. At the anterior end, two slender tentacles, with knobbed ends, project from under the mantle-fold. Two large 'oral lappets,' the 'cephalic lobes' of Pelseneer ('01, p. 20), overlie the mouth region; in creeping they are constantly in slight contact with the substratum. The margin of the encircling mantle-fold is serrated; certain of the marginal projections rep-

resent the location of large mantle-glands, which give rise to a repugnatorial secretion; there are fourteen of these glands in O. floridanum, seven on either side.

Although at about the time of low water Onchidium is often abundant upon shore rocks (cf. Semper, '81; Eliot, '99), and in many places even conspicuous, during high tide it is invisible. It never wanders above high-water mark nor beneath the water level. In these respects it differs sharply from a much smaller species (Onchidiella) of intertidal habitat also found at Bermuda (Arey and Crozier, '19 a, p. 163), the latter creeping about when covered by the sea, but being sheltered within dead Serpula tubes, barnacle shells, and the like during low tide. Eliot ('99) has briefly referred to the occurrence of O. tonganum on the tidally exposed reefs at Apia, Samoa. O. floridanum also lives upon more or less isolated rocks and islets, within Great Sound, but does not occur on the Bermuda 'coral' reefs, which are not exposed at low water.

We were soon struck by the fact that sometimes, even when the tide was not high, no Onchidia were procurable in places known from preceding and from subsequent observations to be thickly populated by them. There is in fact a very exact relation between the appearance of the Onchidia upon the rocks and the state of the tide. This relation involves some curious and precise 'homing' habits, of a kind hitherto unsuspected among mollusca (Arey and Crozier, '18).

Onchidium lives in communities numbering a dozen or more individuals, with pocket-like 'nests' in the eroded shore rock. The openings to these nests are as a rule very inconspicuous. The mollusks creep out of their nest only when the tide has a little more than half ebbed, that is, about 2 to 2.5 hours before low tide. For any particular nest the time of emergence depends upon the nearness of the nest to the high-water mark. Those Onchidia living in nests located nearest to the high-water level appear in the open sooner than do those situated farther down. It is the actual position of the cavity of the nest itself, in relation to the tidal level, rather than the location of its external opening, which regulates the moment of emergence.

A common habitat of Onchidium is on isolated rocks which are more or less completely submerged at high water and are covered by a yellowish-brown felt-work of algae. On such islets, and on more extensive protected shores of similar appearance, numbers of tiny crevices are almost invariably lined with a layer of Modiolus,[3] and these frequently contain a passageway to an Onchidium nest. The eroded cavity in the limestone forming the irregularly shaped nest is sometimes of the bigness of a man's head, though usually much smaller. The external openings of the passageways are quite inconspicuous, for they are not only small, but they are further masked and partially choked by the growth of Modiolus. It is astonishing through how small an opening an Onchidium can slowly make its way, as it insinuates itself into the tiny spaces between the mussels. An individual that is 5 mm. high when normally creeping can squeeze through the space between the edges of two glass slides held 1 mm. apart. In nature the process seems even more startling. When a group of Onchidia emerges from the nest the individuals appear one at a time in continuous series.

Colonies were also found established at sheltered spots where loose stones were held together by red clay, the nest being here a deep crevice between two stones. This type of habitat is less common than that afforded by eroded limestone.

Following the emergence from its nest, a colony of Onchidia wanders in various directions over the rock. Some individuals may creep a meter from the nest. They remain exposed for a certain length of time, and then, *simultaneously, return directly to the nest from which they came.*

The individuals emerging from one nest sometimes become more or less scattered, separated, and even somewhat mingled with others derived from other nests. Among the components of any one community, however, the coincidence of the return to the home nest is in most cases, if not indeed in all, remarkably

[3] This Modiolus is sometimes found underneath small stones and on the under sides of rock slabs, where numbers of stones occur piled together. In such darkened situations the color of the mussel is not black, but, on the contrary, retains the reddish-brown cast of the juvenile shell.

close; one is tempted to compare this rather startling exhibition to the effect of a thunder-storm in causing human families to retire to their respective homes. On the other hand, the colonies which, in any given place, are the first to appear are likewise the first to retire, so in this way a separation of the different communities is effected, favorable to their exact observation. Independent study has convinced us that there is probably no 'mixing up' of the individuals from different communities; in fact, we have never seen an instance in which an individual coming from one nest and carefully watched during the whole of its unmolested perambulations, failed to return to its original home.

The process of return to the nest has a highly determinate aspect. The return course is direct and as straight as is permitted by the physical imperfections of the substratum. In creeping back to the nest an Onchidium may move toward the sun, although if removed from the rock and immediately placed on a glass plate it will be found negatively heliotropic. Heliotropism has nothing to do with the direction of normal creeping (Crozier and Arey, '19 c).

In the immediate vicinity of the entrance to the home cavity, the Onchidia frequently make use of a natural groove in the rock. In the case of small colonies, all the individuals may utilize this trail. The members of larger colonies, however, may simultaneously approach the nest from several widely separated points. Arriving at the entrance to the nest, they crowd about it in an absurd orderly fashion, and without restlessness 'await their turn' to enter. Owing to the fact that the opening of the nest is usually quite small, several minutes may be required for an individual to insinuate its body into the opening. Hence the disappearance of the whole colony within the nest usually occupies some time. Once, however, they have collected about, or upon, the mass of Modiolus which commonly surrounds or even partially occludes the entrance to the nest, the Onchidia, because of the similarity of their coloration to that of Modiolus, may readily be overlooked.

The following notes of one emergence of the Onchidia inhabiting a section of Little Agar's Island will give a sufficient general idea of the phenomenon, which we have repeatedly observed:

July 6, 1914. A cloudy, but fairly bright day. Low tide at 11:30 A.M.

9:57 A.M. A few (4 or 5) Onchidia 'out,' others in process of emergence.

10:02 About a dozen out. Many at once begin to climb straight upward. Others wander in devious paths; if on a flat rock-shelf, may start toward the water, but do not actually go downward.

10:12 In one cove on S. E. side of island 24 individuals seen. Over 100 seen on flat rocks at opposite side of the cove, to the westward; at 10:02, only 1 or 2 were to be seen here.

10:17 A few stragglers are still appearing.

10:32 A few (those which were the first to appear) are beginning to retire.

10:37 Some Onchidia from nests lower down than those previously concerned are beginning to come out. For these, the tide is as much lower than the nest as it was in the case of those first appearing.

11:02 Excepting a few, the Onchidia seen out at 10:12 have all returned. At 11:09, only 3 of this first group remained out.

11:42 A community seen coming out at 10:30 has now completely retired.

12:20 P.M. About 10 Onchidia, as nearly as could be determined, are now to be seen on the entire island.

The foregoing record illustrates some of the general features of the appearance of the Onchidia upon the rocks and of their return to their nests. Several further records may be cited which exhibit the remarkable synchronous character of the return to the nest on the part of the different individuals of a colony, even when these individuals may be separated from one another by a relatively considerable distance. These records are typical of the many observations made upon this point, and no facts discordant with them have ever been encountered.

July 2, 1914.

9:30 A.M. A bright sunny day. Only a very few animals to be seen on Little Agar's Island. Low tide occurred at 8:00. [Doubtless the few animals visible were late comers from nests near low-tide level, but no note was kept of this.]

9:40 On a rock 3 Onchidia, in a group, were seen creeping back and forth; one of these wandered two feet away from the others.

9:55   The two animals turned and went directly back toward their nest. The solitary one, two feet away, turned back at about the same time.

10:06   All three reached entrance of the nest, by a straight route in each case.

April 16, 1918. Dyer Island.

Low tide 5:30 P.M. Several rocks some feet from the main shoreline, on the southern side of Dyer Island, afforded convenient stations for study of Onchidium colonies occurring more closely grouped, on the whole, than was the case on Little Agar's. At the top of one of these rocks there was a single colony, well removed from the closely clustered nests around the lower edge of the rock. At 4:46 the members of this topmost group began to emerge; by 4:53 the whole colony, numbering 17 individuals, 3 to 13 mm. long, had emerged. They scattered in various directions over the rock; some going 70 cm. away from the nest entrance. No animals from other nests became mingled with them. By 5:37 one Onchidium of this group had turned and begun to creep toward home; at 5:48 the last one of the 17 had done likewise, the whole number finally arriving at the entrance to their nest by 5:55.

Hirasaka ('12) has noted that the Japanese O. verruculatum Cuv., though easily obtainable at low tide, seemed at high water to have 'disappeared.' He also records that this species is not seen, even at low tide, during stormy weather, and suggests that the animals retire to clefts in the rocks. This corresponds to the behavior of O. floridanum, with the difference that in stormy weather this species does not emerge from its nests on a given bit of shore if the latter be exposed to wind or surf. Rain or an overcast sky has a negligible effect, if indeed any at all, upon the emergence of the Onchidia. It is stated that O. verruculatum is most abundant from April 15th to October 15th, and Hirasaka suggests that this species, like other pulmonates, passes through a period of hibernation (also Fujita, '97).

O. floridanum, in the warmer Bermuda region, does not hibernate although some of our field notes suggest that on some of the colder winter days the animals may fail to emerge. Further observations would be necessary to settle this point.

The intertidal rock crannies inhabited by Onchidium celticum were carefully described by Joyeux-Laffuie ('82), who detected also the fact that these animals "abandonnent leur retraite en moyenne une heure ou une heure et demie après que la mer a

commencé à baisser," and that they again seek shelter in these cavities with the return of high water. But the peculiarly significant fact that each Onchidium returns to a particular crevice, Joyeux-Laffuie did not discover (if indeed it occurs in his species). He pointed out that in O. celticum, as in our form, the attachment of the foot to the substratum is feeble, so that the snail if exposed under water would find it impossible to retain its footing. He states also that "Le moment où elle sort des fentes des rochers et celui où elle s'y refugie sont très variables, suivant la température," this species rarely emerging in winter; moreover, "par un temps couvert et humide, elle se promenent beaucoup plus longtemps que par un temps clair et sec." For O. floridanum we can agree that temperature is probably a factor in this matter, but the degree of atmospheric humidity has seemed quite insignificant. According to Bretnall ('19), two species of Onchidium observed by him in Australia did not show the possession of 'homing habits.' No details are given.

Further data regarding the curious homing behavior of Onchidium, together with such analysis of the situation as may be attempted on the basis of our inquiries, are best deferred until something has been said concerning the sensory reactions of Onchidium. Some additional features of the creature's natural history must first be presented.

Locomotion

The mechanism of the pedal creeping of Onchidium has been noted by several observers (cf. Parker, '11; Olmsted, '17a). As in the case of many other pulmonates, progression is accomplished by means of pedal waves originating at the posterior end of the foot and coursing anteriorly (direct waves). Ordinarily but one of these waves, extending across the whole width of the foot (monotaxic), is present on the foot at a time. Another wave is initiated just as its predecessor reaches the anterior end of the foot. Sometimes two waves are visible at one time, the second having been commenced just before the disappearance of the first. A wave causes the posterior extremity of the foot

to be contracted anteriorly for a distance of 2.5 to 3 mm.; the posterior end, moving forward more quickly just before the termination of its 'step' than at the beginning, is then attached; as the wave begins to move anteriorly, the animal appears to brace itself against its point of attachment, the posterior end of the foot spreading out against the substratum and seeming as a whole even to move backward slightly. The pedal wave—as may readily be seen either when Onchidium is creeping on a glass plate in air or when placed upon its dorsum so that righting movements are begun—represents an area of the foot lifted out of contact with the substratum (cf. Olmsted, '17a, p. 235). When an Onchidium is placed on its back, wave movements appear on the foot, and are somewhat magnified as compared with their normal size; inspection of the waves produced in this way shows, under the binocular microscope, that the posterior zone of the wave concavity is undergoing longitudinal contraction in the anterior direction, as it should according to Parker's ('11) view of the mechanics of progression in such cases. The pedal wave traverses a foot surface of 13 to 14 mm. length in about 5 seconds (i.e., at a rate of $16 \pm$ cm. per minute). The rate of progression of adult Onchidia, on a smooth surface, is found to be approximately 5 cm. per minute, agreeing with that calculated from the preceding data regarding the frequency of the waves (20 per minute) and the distance observed to be transversed (2 to 5 mm.) as the result of a single wave (cf. Peyréga et Vlès, '13). Like most gastropods, Onchidium creeps only in the anterior direction; its creeping appears incapable of reversal.

Onchidium creeps over substrata of varied texture, such as bare rock, corraline algae, or felted enteromorpha, and while entering or leaving its 'nest' passes over groups of Modiolus presenting a sharply serrated surface. In agreement with the possibility of the mollusk's living upon such surfaces, it is found that the foot does not serve as a hold-fast through its action as sucker. To some extent, as in Chiton (Arey and Crozier, '19 a), the mantle serves as a hold-fast. If the animal be touched, or shaded (vide infra), the mantle is quickly applied to the rock surface, very much as that of Chiton is. This reaction is inter-

rupted if the dorsum of the animal is pinched or prodded, since the periphery of the mantle with its repugnatorial glands is then elevated and caused to bend toward the focus of irritation. The animal nevertheless continues to adhere with some firmness to the rock. The major share of this adhesion is apparently due to slime, as in other pulmonates. Onchidium can maintain a position upside down on a glass surface when less than one-third of the foot is in contact; it can creep over a crack between two glass plates; it creeps undisturbed over a hole in a glass plate, the sub-stance of the foot being pressed firmly into the vacant space. The adhesion of the foot is due, then, in the first place, to the close contact brought about between the foot and the rock or other surface, and, secondly, to the slime which the foot secretes; possibly local suction on the part of minute areas of the foot is also concerned, as in Chiton, although this cannot easily be tested. Usually, if not invariably, the attachment of the foot can begin at its anterior end only, although the animal may for some minutes maintain its weight by means of adhesion through the posterior third of the foot surface alone.

When rolled over on its back, an Onchidium usually begins soon to right itself. Some individuals, in the laboratory, are quite inactive, however, and will not begin to right themselves for a long time. If turned over while under water, an Onchidium seemingly active in other ways may remain on its back for sev-eral hours, much longer than when in air. In righting, pedal waves of considerable amplitude are formed and continue to pass over the foot until righting is accomplished. When first dis-placed, the animal tends to curl up like a Chiton, but soon stretches out again. The anterior end is twisted and contracted on one side until the anterior portion of the foot can be attached. Righting then proceeds as the attachment of the pedal surface progresses toward the posterior end, so that when half attached the body of the Onchidium is twisted through an angle of 180°. Under ordinary conditions an Onchidium will right itself in 15 to 30 seconds (mean, 25 seconds). It should be noted that the back of an Onchidium is strongly arched, so that when dis-placed from the rock, and caused to 'curl up' with its fringe of

poison glands projecting, the body is admirably shaped for rolling over to one side or the other, even before twisting is begun, the form thus tending to facilitate righting. We have never seen an Onchidium in nature disturbed to an extent sufficient to bring this behavior into play. It is unlikely that they are ever dislodged by wave action, even though the force of attachment through the foot is not great, since they do not creep upon the exposed rocks under water; nor do they come out of their 'nests' at low tide when there is a stormy wind; nor do they on the whole inhabit places where wave action is severe, but rather the reverse.

In nature, Onchidium must spend a good part of each day under water, sometimes several days continuously. Certain of the nests may entrap a small amount of air as the tide rises, but this cannot be the case with most of the nests. In aquaria, these animals will live for at least two weeks under sea-water, without visible impairment. In several instances groups of them were forced to do so by being placed upon a stone suspended by a string under water. A group isolated in this way stays during daylight hours, and also for the greater part of the night, on the under (shaded) side of the stone.

When beneath the water surface an Onchidium tends to creep more slowly than when in air, and remains for long periods 'at rest,' with girdle depressed, dorsum arched, tentacles retracted. While creeping under water the tentacles are never so far protruded as they characteristically are in air. As already noted, the attachment of the foot to the substratum is not very firm in Onchidium; it is, however, quite sufficient to enable the creature to creep on the under surfaces of stones in air as well as under water.

Onchidium emerges from its nest at low tide even though rain may be falling heavily, provided no wind is blowing strongly on the bit of shore concerned. It is therefore interesting to determine the toxicity of rain-water for Onchidium, especially since the porous rocks containing the nests of these mollusks must frequently permit the seepage of rain-water into the Onchidium shelters. Experiment showed that an Onchidium placed

in 200 cc. of rain-water would live in some cases for 4 hours (at 27°C.). When first immersed the animal partly rolls up, lying on its side or back, and it so remains until dead. If stimulated by touching, it momentarily writhes about, then returns to quietness. A fair degree of sensitivity is retained for at least 3 hours in rain-water. The intertidal Chiton tuberculatus is similarly resistant (4 hours), but is in addition protected by the completeness with which the girdle can exclude fluids from contact with the animal's soft tissues (Arey and Crozier, '19). The nudibranch Chromodoris is killed by 45 minutes' immersion in rain-water (Crozier and Arey, '19b). Resistance to rain-water may clearly be of bionomic importance, but that it has originated adaptively is in no degree certain; more probably, it depends upon the organization which Onchidium has inherited from ancestors among the land pulmonates (Perrier, '17).

Certain phenomena of coloration in O. floridanum are not without interest, especially in view of the fact that there is present in this snail an active system of repugnatorial glands. We have recently published some discussion of this matter (Crozier and Arey, '19 a), and need refer here merely to the chief points involved.

At Bermuda this species exhibits two fairly distinct types of pigmentation, a pale type verging upon dull olive-yellow, and, much more abundant, a type of dark blue-black appearance. These two kinds of coloration are found in other species of Onchidium (cf. Eliot, '99; Dall and Simpson, '01) and in the related genus Onchidiella. The lightly pigmented type is often concealingly adjusted to its background, but not in every case. The color of the dark variety might also be considered a concealing match for the mussels[3] which cluster about the entrance to its nest. But no correlation can be established between specific substrata and the pigmentation of the Onchidia which creep over them at low tide. In view of this fact and of the further observation that the mud-encrusted slime pellicle investing the back of Onchidium is commonly removed in mechanical fashion as the snail creeps out of its nest, whereas if the pellicle should

remain it would add decidedly to the creature's homochromicity,[4] it is important to note that the powerful repugnatorial mantle-glands are found developed to an equal degree in Onchidia of whatever variety of pigmentation. Bretnall ('19) states that O. dämelii and O. chameleon exhibit "the chameleon-like property of changing their colors, especially when disturbed" or placed upon a different background. No basis for such color modification is known, nor is one described by Bretnall; nothing of this sort occurs in O. floridanum.

These facts are incompatible with the notion that the coloration of Onchidium involves adaptive restraint. Some reasons have been given (Crozier and Arey, '19 a) for regarding the coloration of an Onchidium as the result of genetic factors primarily. It is therefore noteworthy that the breeding habits of this snail may provide a mechanism for the perpetuation of a racial type, lightly pigmented, which probably would behave as a recessive in crosses with dark-hued forms. Onchidia are 'simultaneous' hermaphrodites, exhibiting reciprocal insemination (cf. also Joyeux-Laffuie, '82). Joyeux-Laffuie states that O. celticum conjugates during its periods of emergence upon the rock. We have never seen this in O. floridanum, and believe that copulation occurs within the nests. Even if it should occur in the open, however, each colony is in large measure prevented by its habits from mingling with the members of other communities, so that an appreciable degree of inbreeding may safely be postulated. Eggs are deposited during July within the nests, attached in pearly masses to the upper surfaces of the rock cavities, and the creature which emerges from the egg membrane has already the form of an adult.

The intimate physiology of the peripheral glands, referred to as 'repugnatorial glands,' will be considered in a subsequent paper. It is pertinent, however, to mention here some observations upon their use in nature.

Semper ('81) regarded the glands as of service in warding off the attacks of certain intertidal fishes. According to Semper,

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[4] It may be pointed out that the non-removal of the slime coating of the snail's back might interfere with dermal respiration.

the shadow of an approaching fish induced the discharge from the glands of a liquid spray which drove off the fish. This notion was extended by Semper so as to provide a mechanism explaining the development of the mantle-eyes of Onchidium. He considered that the distribution of those species of Onchidium possessing mantle-eyes coincided with that of the intertidal blenny Periopthalmus. This remarkable fish skips along the beach zone laid bare by the falling tide (cf. figure in Hess, '12), and was said by Semper to prey upon Onchidium. The testimony of later naturalists does not favor this view. Periopthalmus lives upon mud flats and frequents the margins of mangrove swamps (Murray, p. 489; Eliot, '99), whereas Onchidium lives upon rocks and along the edges of reefs, where the blenny is not found (Eliot, '99). Periopthalmus, moreover, feeds on arthropods (Murray, loc. cit.). Australian species of Onchidium possess mantle-eyes, and in regions where no Periopthalmus-like fishes are known (Bretnall, 1919). Although Onchidium is quite sensitive to shading (Crozier and Arey, '19c), O. floridanum does not discharge its poison glands when stimulated by a sudden decrease of light intensity; tactile excitation is the form of stimulation pre-eminently successful in eliciting discharge of the glands. The dorsal surface of O. floridanum is, however, the part sensitive to shading, while one of the most conspicuous features of the animal's response to shading is the retraction of the tentacles, the latter organs being, nevertheless, themselves devoid of direct excitability by shading.

Neither Periopthalmus nor any other fishes of analogous habit occur at Bermuda. It is improbable that fishes are able to enter the majority of the Onchidium nests, because of the minuteness of the entrances. It seems likely that it is in air, rather than when under water, that the glands are most effective. The gland contents are discharged in a stream which in air breaks up into a fine, almost invisible, spray, which may be thrown as far as 15 cm., or about ten times the length of the Onchidium. Under water, long threads of secretion are expelled, which do not form a spray and fail to travel more than a centimeter or so from the apertures of the glands. Tactile stimulation of the dorsal

surface of the mantle is the kind of excitation most effective for gland discharge. Among the animals noted as frequenting the Onchidium zone were included: isopods (Ligia, especially), crabs (Sesarma, Panopeus, Porcellana, in some places Pachygrapsus, and an occasional Portunus), Chiton tuberculatus, Coscinasterias (during its breeding season—January to February— often left above low water, in a depression between rock slabs and in similar places), and the mud-dauber wasps (Polistes), which gather moist silt from cool, shaded, intertidal spots near the entrances to caves. Unequivocal instances have been found in which crabs, isopods, starfish, and wasps came into contact with Onchidium; in most, if not indeed in each of these instances there occurred immediate moderate discharge of the glands, followed by the retreat of the animal from the Onchidium. It must not be supposed that these creatures were endeavoring to devour the Onchidia; rather, it seemed important for the snails to avoid being accidentally pushed off the rock into the water, for, as previously noted, Onchidium does not adhere with any great firmness to the algae-covered rock. When purposely pushed off, into the water, an Onchidium is not able to return to its nest. It is entirely probable that most of the creatures accidentally touching one of these snails would have retreated even in the absence of the repugnatorial secretion, but the importance of the discharge is nevertheless clear.

The gland secretion was obtained in a 'pure' state by holding a glass slip over the back of an Onchidium stimulated mildly, in air, by means of faradic shocks. The glands individually turn their apertures dorsalward and their axes converge in such fashion that the several discharges impinge very nearly at a single point immediately above the site of stimulation. This conspicuous accuracy involved in release of the gland content is a noteworthy feature of the use of the glands. Small bits of crab- and mussel-meat were smeared with the secretion and were found to be vigorously rejected as food by sea-anemones, star-fish, crabs, and fishes, including forms which could not by any possibility have previously encountered this material, as, for example, Fundulus from landlocked brackish ponds at Ber-

muda (Crozier, '19 b). When received upon one's tongue the repugnatorial spray is found to sting like wild mustard, and with considerable persistence (Crozier and Arey, '19 a).

It is not likely that many creatures able to inflict damage upon Onchidia can gain access to them while they are concealed within a 'nest' and covered by the sea. Among those which need be mentioned in this connection one of the most interesting is the curious littoral chilopod Hydroschendyla.[5] These rare forms occur at Bermuda between tides, in crevices and within the muddy interstices of much-eroded sandstone blocks. Like their geophilid relatives, they devour annelids, for they have been uncovered in the act of biting into the sides of Leodocid worms which occur between the aeolean strata.[6] On several occasions Hydroschendyla has been obtained within Onchidium nests when these were chiseled open. No indication was had, however, of either symbiotic or predatory connection between these forms—their association seemed entirely accidental.

SENSORY RESPONSES

1. Mechanical excitation

The responses of Onchidium to tactile excitation are of some diversity, depending upon the part activated. The surface of the foot responds by attachment when brought into contact with a surface sufficiently large; the glands upon the periphery of the mantle become erected and release their contents when the mantle is stroked or pinched, but otherwise the reactions to touch are of the more or less local and negative (withdrawing) type. General mechanical activation by water currents induce negative rheotropism. A kind of anemotropism, involving the

[5] We are indebted to Dr. R. V. Chamberlin, Museum of Comparative Zoölogy, for the identification of this chilopod. (Cf. Chamberlin, 1920.)

[6] Geophilus has been figured wrapped spirally about the body of an earthworm which it had begun to devour. Hydroschendyla, however, seems merely to bite into the body of Leodocids, whereupon the worms conveniently autotomize at that place, the anterior end creeping away while the centipede sucks the juices of the abandoned tail. It is interesting to observe that allied Schendylids are known to frequent caves (Ribaut, '15).

stimulation of the tentacles, occupies a well-defined place in the bionomics of Onchidium. Geotropism is not well defined and reactions to vibratory stimuli are but poorly represented.

When the back of an Onchidium is momentarily touched, a slight local depression is formed. The dorsum is not very active to a single light touch. If the dorsum be 'scratched,' however, or stroked several times in succession with a blunt point, the particular area affected becomes to some extent contracted; but the most obvious response is from the margin of the mantle— the mantle-fold is erected, forming a saucer-like rim about the body, so that the now erected repugnatorial glands come to point in a general way toward the spot irritated.

The marginal zone of the mantle is much more sensitive to touch than is the back of the animal. If the anterior end of a creeping individual be lightly touched, the tentacles and oral lappets instantly retract, the mantle-fold is depressed to the substratum, the back of the animal becomes strongly arched, and locomotion ceases. Three or four gentle stimulations in succession are required to induce the completion of this form of response, but a single touch is sufficient to bring about the expression of its initial phases. Almost immediately after the stimulation has ceased, the head and tentacles are protruded from beneath the mantle and locomotion is resumed. If the animal is, to begin with, not creeping, but quietly attached in its characteristic attitude (the head and tentacles being withdrawn, the body then appearing oval or circular in outline), a touch causes the edge of the mantle to be retracted and the back more decidedly arched. Stimulation of the posterior end of the mantle of a resting Onchidium causes the part affected to be drawn forward and curled under the body. In a creeping individual, if the posterior end be touched several times in succession, the whole posterior third of the body is contracted, so that in outline, seen from above, it is pear-shaped; but the locomotion does not cease, and the animal continues to creep with its posterior part contracted in this way for several minutes. The peripheral edge of the mantle is quite sensitive to touch, reacting by local retraction.

The ventral surface of the projecting portion of the mantle when stimulated undergoes reactions of a sort similar to the preceding at the anterior or the posterior extremity. At the sides of the body, the mantle locally bends ventralward toward the substratum when its lower surface is activated. If the periphery of the foot is touched, the substance of the foot is puckered away from the source of stimulation, and the mantle is depressed at this point. The ends of the foot are more sensitive than its lateral edges. Stimulation of the end of the foot causes the animal as a whole to contract, arching its dorsum. The ventral surface of the foot, which may be studied by allowing the Onchidium to creep over a gap between two glass plates, reacts negatively to the tactile activation of a blunt-pointed instrument, and the lateral margins of the foot on both sides at the level of stimulation contract locally toward the median line.

The tentacles and the oral lappets are the parts most sensitive to touch. A tentacle stimulated at its tip or at a point along its stalk is quickly rolled inward, glove-finger fashion, like the tentacle of a snail; it is then re-extended more slowly. The response is unilateral. Unsymmetrical tactile excitation of the tentacles may be used to direct the path of locomotion, as was attested by the fact that an Onchidium moving away from a source of light could be made to alter its direction by repeatedly touching one tentacle; the animal turns away from the stimulated side.

Activation of the anterior portion of the mantle-fold at one side results in the contraction of the tentacle, of the oral lappet, and of the head as a whole on the homolateral side; the mantle-fold is itself at the same time locally depressed. More intense stimulation or a light touch repeated four to six times leads to a similar response from the opposite side of the head as well.

If a tentacle be very lightly touched, it alone responds; if somewhat more strongly stimulated, the homolateral oral lappet is also involved in the reaction. An oral lappet, however, will respond repeatedly without the homolateral tentacle being implicated. The decided parallelism between these peculiarities— homolaterality of response and irreciprocal conduction between

tentacle and oral lappet—and the corresponding behavior of the analogous organs of a nudibranch, such as Chromodoris (Crozier and Arey, '19b), should be noted here.

The high tactile irritability of the oral lappets was noted by Joyeux-Laffuie ('82, p. 311), who described also the richness of their epithelial innervation and the manner in which, as we have already described, these organs are constantly engaged in 'feeling over' the substratum during creeping.

Onchidia placed in a trough through which a gentle current of sea-water is maintained soon become oriented by the current and creep with it. It is not altogether certain to what extent such orientation may be a purely passive one, for to an under-water surface the foot of Onchidium is not very firmly attached, so that, as the anterior end of the foot is sometimes lifted, this part of the body may be mechanically swung around with the current.

A location affording on a calm, sunny day several hundred Onchidia may be searched in vain for a single one if a strong wind be blowing from such a direction as to impinge upon this particular stretch of shore, while at other places, sheltered from the wind, the usual complement of feeding snails is seen at low water. With the idea that perhaps the explanation might here be found for the non-emergence of Onchidium during stormy periods, individuals were taken from the laboratory stock and allowed to creep upon a horizontal slab of stone freely exposed to the wind. They became promptly oriented so as to head away from the wind. The tentacles were sharply retracted when first struck by the breeze, then subsequently slightly extended. When the tentacles were removed by a quick snip with scissors, these animals were not longer oriented by the wind. If but one tentacle was removed, an Onchidium was found to go through a sort of 'circus movement,' tending strongly to bend toward the unstimulated (non-tentacled) side. The tentacles seem therefore to serve as anemotropic receptors. In agreement with this a number of rocks containing Onchidium colonies were found to have their upper surfaces and windward sides free of exposed Onchidia, whereas the protected leeward

faces of these stones bore many feeding individuals. In one case three of these had their tentacles removed, and when placed in exposed situations crept into the breeze without hesitation. It is probable, however, that a still stronger wind would orient detentacled Onchidia. (No tests were made of the orientation of detentacled snails in a water current.)

It should be mentioned here that in the tips of the tentacles there are found considerable numbers of nerve cells; the possibility is suggestive that they may be important of anemotropic sensitivity.

With O. celticum, Joyeux-Laffuie noted that certain stretches of shore might harbor many hundred Onchidia, whereas nearby stations exhibited few or none; he did not believe that the character of the food supply was instrumental in determining the erratic character of this distribution, but gave no evidence bearing upon the real explanation. Similar facts are quite evident at Bermuda, and we regard it as clear from our own studies that the degree of exposure to wind, and possibly surf, is a preponderating factor in the matter. Protection from the force of wind and surf is essential, and at stations notably deficient in this particular no Onchidia are found.

Onchidia placed at the bottom of a battery jar soon climb its side, and generally do so in a straight line perpendicular to the ground. If the dish be covered they will accumulate at the top, some remaining on the vertical surface, others upside down on the glass cover; particularly during the first hours of such a test it is rare to see even one individual moving downward again, and the whole group will usually stay at the top for days. In the absence of a cover, however, the same animals readily creep over the edge and on, downward, to the table. Onchidia placed on a glass plate seemed on the whole to be negatively geotropic, since in many instances appropriate reversals in the direction of creeping could be induced by turning the plate; such results were not always forthcoming, however. The natural situations of the Onchidium nests involve normally some degree of upward or downward creeping, since not infrequently the opening of a nest will be on an almost vertical surface. But aside from the

fact that the snails never creep down to the actual water level, they do creep upward or downward with seeming indifference. Only a limited degree of negative geotropism may therefore be postulated for Onchidium.

Vigorous vibratory mechanical stimulation serves to interrupt the creeping of an Onchidium, but neither the sharp tapping of a glass beaker containing some of the animals nor forcible blows struck upon natural rock bearing them leads to any more pronounced form of response. Although the snail may altogether cease creeping for some minutes when disturbed in this way, the tentacles are not retracted, nor is the mantle-fold depressed.

2. Photic excitation

The data presented in this section were all secured under laboratory conditions. In nature the heliotropism of Onchidium is inhibited (Crozier and Arey, '19c), although its reactivity to shading is quite pronounced.

Onchidia gather in groups on the side of aquaria away from the light, and, once there, in the majority of cases stay on that side. Diffuse daylight and brilliant sunlight induce the same form of response, which obtains whether the animal is in air or under water. After reaching the side of the container farthest from the light, they usually continue to creep upward to the water edge, where a brief halt commonly ensues, and then on up. To a source of unilateral horizontal light, an individual orients sharply, precisely, without 'trial' movements. Having oriented, the animal proceeds to move in a straight line away from the light source.

In a dark room the surface of Onchidium was explored with a minute and fairly intense beam of light (cf. Patten, '15). The diameter of this beam was about 0.3 mm. The anterior end of the mantle was found the most sensitive part of the creature's surface. Even with this minute source of stimulation it was possible to make the animal move in any desired direction by appropriately placing the spot of light upon one side of the anterior end of the mantle. The photic excitability of the posterior part of the mantle was somewhat lower than that of

the anterior end of the mantle. The stimulation of the posterior region of the mantle led, however, to a type of response not seen when the anterior portion was activated. When the light-spot was applied to a point anterolateral with respect to the mid-posterior point, the anterior end of the Onchidium was swung sharply toward the stimulated side; when the body was then, immediately afterward, straightened, the stimulated spot was swung out of the region of activation. The body of Onchidium is not readily twisted sideways, and it seems that when such twisting does occur it is always initiated by the anterior end, and is of such a character as to contract the animal on the side of the stimulated spot. This spot must, however, be located on the posterior half of the body. In the case of photic irritation, the resulting maneuver is very efficient in withdrawing from activation a given stimulated posterior part, and seems purposive; but it appears to be the only form of reaction possible under the circumstances. At the anterior end of the mantle, however, the reaction elicited by a spot-light is a more purely directive one, leading to less pronounced differential body contraction, but to more vigorous locomotor movements.

The whole mantle dorsum is photosensitive. When one point was activated by the spot-light, a puckered depression quickly appeared and extended as a furrow transversely across the animal's back.

Stimulation of the anterior end of the foot or of the head region (beneath the mantle) was, so far as could be detected, without directive effect.

The tentacles, also, seemed themselves to be non-reactive to illumination, nor when stimulated either by the spot-light or by concentrated sunlight did they induce directive locomotion.

The tentacles are not important for orientation by light. Onchidia from which the tentacles have been amputated still orient away from the light as sharply and as promptly as with the tentacles intact.

These experiments suggest that the dorsal mantle surface contains the only photoreceptors important for phototropism. This is confirmed by the following experiment:

Animals from which the tentacles had been removed had the right or the left half of the dorsal surface smeared with a mixture of lamp-black and vaseline. When a narrow beam of sunlight was allowed to fall on the blackened side of the body, no response of any kind was observed. When the unpainted side was exposed to the light, how-ever, these animals behaved like normal ones—they oriented precisely and always toward the darker side.

The distribution of photic irritability, which thus seems to be confined to the dorsal surface of the mantle, is sufficient to show physiologically that differentiated receptors are concerned in the reactions of Onchidium to light. The exclusive photic irritability of the dorsal surface of the body is important in connection with the well-known mantle-eyes present in some members of this genus. Two views have been advanced con-cerning the phylogeny of these eyes: (1) that under bionomic stress the eyes have developed from some less specialized integu-mentary photoreceptors, this being the essence of Semper's idea, and (2) that eyes of this type were early developed by the primitive Onchidium stock, and have subsequently become in some species lost or rudimentary (cf. Stantschinsky, '08). In O. floridanum there are no differentiated mantle-eyes, and, although tentacular eyes are present, no evidence has been forthcoming to show that they actually play a part in the creature's activities, or indeed that they are in any degree photo-sensitive. A physiological analysis of the photic sensitivity of Onchidia possessing undoubted mantle-eyes should afford some data valuable in this connection, but has yet to be made.

When the light falling upon an Onchidium quietly creeping in air is suddenly decreased, the tentacles of the mollusk are quickly and forcibly withdrawn beneath the mantle, the head is retracted, locomotion stops, and the mantle is lowered into con-tact with the substratum. In the case of a snail which has for some time been undisturbed in this way, a very slight decrease in light intensity induces response of almost maximum ampli-tude. In any event, the snail quickly resumes the attitude it had previous to stimulation, even though the reduced illumina-tion be maintained. If a shadow be cast on the anterior end only, the head is sharply withdrawn, locomotion ceases, the

mantle-fold being promptly depressed; at the posterior end, when shaded locally (from behind), the posterior part of the body is likewise contracted and depressed, but the contraction of this portion of the body is not so pronounced as when, in the case of anterior shading or of the shading of the whole body, the anterior part of the body seems to be drawn backward.

No reaction follows the shading of the tentacles alone, but if the anterior edge only of the mantle be shaded, a normal reaction follows. Onchidia from which the tentacles have been

TABLE 1

Showing the course of exhaustion of the response to shading in three individuals (Onchidium) in bright sunlight; shaded at three-minute intervals

NUMBER OF STIMULATION	RESPONSE		
	Animal 1	Animal 2	Animal 3
1	Complete reaction	Complete reaction	Complete reaction
2	Complete reaction	Only tentacles retracted (slightly)	Weak total response
3	Reaction mostly from the tentacles	Just perceptible response of tentacles	Good complete reaction
4	Tentacles retract slightly	One tentacle partially contracted	Tentacles only, retracted
5	Tentacles retract just perceptibly	One tentacle slightly bent away	Tentacles only, retracted
6	One tentacle caused to bend to one side		
7	One tentacle caused to bend to one side		

amputated are normally responsive to shading, even within five minutes subsequent to the removal of the tentacles.

Reactivity to shading is quickly exhausted by rapidly repeated activation. In bright sunlight, one or two 'complete' reactions are all that are usually obtainable when the shadings succeed one another at intervals of 3 mm. (cf. table 1). Successive shadings evoke responses gradually less complete.

When the receptivity of the anterior end for shading stimulation is completely exhausted, this part is nevertheless fully responsive to delicate tactile activation. Many tests were

made with the aid of a glass plate upon which there was a small opaque dot of india-ink, thus enabling a small shadow to be cast upon the back of an Onchidium. In a resting animal, if the shadow spot so produced was made to move slightly upon the back of the mantle, a response was usually provoked, in the form of a slight retraction of the tentacles with or without the depression of the mantle.

Under water the tentacles of Onchidium are never extended so fully as when the animal is in air. The response to shading is therefore not so conspicuous when the animal is under water, since the tentacular retraction is not so obvious; otherwise, the behavior of Onchidium when shaded is identical under water and in air.

Onchidium gives no response whatever to increase in light intensity, as such.

Onchidium is but one of a number of animals in which it has been demonstrated that precise negative orientation by light may occur simultaneously with the presence of definite and conspicuous negative responses to decrease of light intensity, reactions initiated by increase of light intensity as such being absent (Euglena, Bancroft, '13; the leech Dina, Gee, '13; holothurians, Crozier, '14, '15; Chiton, Crozier and Arey, '18). Onchidium resembles Chiton and the holothurians especially in the fact that the regional distributions of the two kinds of irritability are seemingly identical. Phenomena of this kind nullify the supposition that the orienting stimulus can originate, for these animals, in the changing intensity of light; because the sense of their only known form of response to changes of light intensity is incompatible with the manner in which photic orientation occurs. The significance of this fact, especially as demonstrated in bilaterally symmetrical animals, seems to have been unduly ignored.

Additional evidence was secured from Onchidia repeatedly shaded at various rates until they ceased to respond to shading at all. The orientation of these individuals in a field of light was in no particular different from that of snails with undimmed reactivity to shading.

If a sharply defined narrow beam of sunlight, about 1.5 times the width of the animal, is reflected into a dark chamber containing an Onchidium, the snail orients and creeps away from the light. Slight deviations bring the animal within the zone of shadow, whereupon it retracts sharply, being thus confined to the light beam. After a few shading stimulations, however, this kind of reactivity is exhausted, a slight deviation puts the animal's anterior end in the shade and the rest of the body follows. Similarly, if an animal be shielded from the light on one lateral half, and then illuminated from behind, it turns and creeps into the shade.

The photic orientation of Onchidium is therefore a purely tropistic process, determined through photochemical transformations localized in specific receptors. In the similar case of Holothuria it has been suggested (Crozier, '15) that the same photochemical system may afford the receptive basis for both the continuous action of light and the sudden decrease of light intensity; final proof for this suggestion cannot as yet be offered.

According to the theory of responses obtainable under these conditions, the musculature upon either side of an unequally illuminated, bilaterally symmetrical animal undergoes differential contraction, resulting in forced movements of orientation with reference to a single source of light (Garrey, '18). With such an organism as Onchidium it might then be conceived that when an individual placed upon its dorsum begins to carry out righting movements, the direction in which righting occurs should be strongly influenced by the relative illumination of the two sides of the body. We have made experiments of this kind with Onchidium. Tests of this point have been made previously, principally with echinoderms. There are reasons for regarding such organisms as the starfish as not well adapted for this purpose. The superiority of Onchidium consists in the fact that it is (minor morphological points aside) a pronouncedly bilateral animal. It is necessary to point out that tactile stimulation of the dorsal surface may complicate such tests. The righting of an Onchidium involves the lateral twisting ventralward of the anterior end of body and foot, so that the anterior portion of the

foot is enabled to attach itself. If illuminated from one side only, Onchidium almost invariably contracts the musculature of the *opposite* side when righting is begun.

We may consider at this point the nature of certain evidence sometimes adduced in adverse criticism of the idea that photochemical transformations are responsible for the activation producing heliotropic movements. The theory of heliotropic movements centers upon the fact of orientation in a field of light; if the light for any reason produces unequal effects in the symmetrical receptive areas, the rates of photochemical transformations will not be the same in these areas "and the rate at which the symmetrical muscles of both sides of the body work will no longer be equal; as a consequence the direction in which the animal moves will change." It has been deduced from such statements that the *rate of locomotion* of an animal, once oriented (or already oriented), should be proportional to the acting light intensity (Dolley, '17).

This idea is not necessarily correct, and it may be pointed out that there are forms for which it can at most have but a limited applicability.

The *locomotor progress* of Onchidium is determined by the succession of transverse neuromuscular waves upon the sole of the snail's foot. These waves traverse the length of the foot at a rate of about 16 cm. per minute, succeeding one another at intervals of approximately five seconds (at 27°C.). The speed of these waves and their frequency are very largely independent of the proximal stimulus, once a certain threshold of activity has been exceeded. But in *orientation* the bending of the body is due to the differential contraction of symmetrically located parietal muscles, quite distinct from those producing the pedal waves. The rapidity of orientation thus depends upon the differential action of the symmetrical halves of the neuromuscular mechanism which controls the lateral bending of the body —upon the degree to which one side is contracted, the other side reciprocally relaxed. It should therefore not prove surprising to find the speed of photic orientation (within limits imposed by the snail's anatomy) proportional to the intensity of the

orienting illumination, but the rate of progression, with orientation established, relatively independent of this intensity.

In agreement with this conception, we have found differences[7] in the speed with which a dark-adapted Onchidium is oriented by light of different effective intensities, but with very slight

TABLE 2

Showing the result of one experiment in which five dark-adapted Onchidia were oriented and allowed to creep in illuminated fields of three intensities of horizontal light. The different intensities were secured by placing the center of the observation stage at distances of 12, 24, and 36 inches, respectively, from a water-screened oil-lamp. At the beginning of each test the light impinged upon one side of the animal, so that 'complete orientation' required a turning through 90°. When orientation had been completed, the rate of creeping over a 6-cm. stretch was measured in the same illumination

ANIMAL	I		II		III	
	Orientation	Locomotion	Orientation	Locomotion	Orientation	Locomotion
1	5.1	7.3	2.5	7.0	1.2	5.2
2	4.8	4.1	3.7	5.3	1.1	6.1
3	5.5	5.2	3.1	6.1	0.9	4.3
4	4.2	6.1	5.3	4.8	1.1	6.0
5	5.6	4.9	2.9	5.0	0.8	4.5
Means......	5.0	5.5	3.5	5.6	1.0	5.2

$$\text{Orient.} = \frac{100}{\text{sec. for complete orientation.}}$$

$$\text{Locom.} = \frac{\text{cm.}}{\text{sec.}}$$

If the rate of orientation were directly proportional to the light intensity, rates under I, II, III, should be in the proportion 9:4:1; actually they are as 5.0:3.5:1. More extensive tests might provide a more complete agreement.

The rate of progression is practically independent of the light intensity.

differences in the speeds of progression under different intensities of light, provided the intensity be sufficient to keep the snail moving continuously. There is undoubtedly, as in Chiton (Arey and Crozier, '19), some correlation between speed of pro-

[7] In making this statement we must for the present regretfully rely mainly upon the results of qualitative experiments. When this work was being done at the Dyer Island laboratory no electric light or other suitable light source was available. Kerosene lamps did not afford light of adequate intensity.

gression and light intensity, but a limit is quickly reached beyond which no sensible increase is possible in the rate at which pedal waves succeed one another upon the foot.   Moreover, it is very probable that the pedal nervous mechanism, as is to some degree indicated by other responses, is 'set off' as a whole, and not bilaterally, by impulses originating in the mantle and passing through the central ganglia.   So long as the photoreceptive mechanism is in bilaterally balanced excitation, the rate of operation of the pedal musculature might then proceed upon the 'all-or-none' principle, uninterfered with by the mechanism concerned in turning movements.   This conception is already well founded for vertebrates (cf. Brown, '14).   The rhythm of the 'scratch reflex' of mammals is independent of the frequency of the exciting stimuli (Sherrington, '06).   More generally stated, the rate of contraction of symmetrical locomotor muscles is very nearly constant so long as the rate of stimulation of the two sides of the animal is the same; the bilateral halves of the central nervous mechanism of control together behave as an independent unit so long as they are not stimulated differentially.   Considerations of this sort make it clear why careful measurements of the locomotor rate of insects oriented in a field of light show no correspondence between rate of creeping and the light intensity (Dolley, '17; Minnich, '19).   One must insist that before an animal reaction can properly be made the basis of quantitative experiments, the nature of the reaction must be carefully reviewed as to its suitability for the purpose in mind; measurements of a phenomenon not itself sufficiently understood are likely to prove a waste of time.   Minnich ('19, p. 406) has also pointed out that light intensity affects the posture of the legs of the bee, but not their rate of rhythmic activity.

The speed and precision of the orientation vary in a characteristic manner with the wave length of the light used.   The experiments upon this topic were qualitative in nature, but were repeated a sufficient number of times to make sure of the essential features of the results, which were as follows: When sunlight or light from a tungsten filament is made to pass through different ray-filters, blue and green lights are very powerful in induc-

ing orientation, yellow light is less so, and red light is relatively
ineffective. In different series of experiments ray-filters of
several kinds were employed; thus in the first series of trials we
used the colored glasses previously described in the work on
Chiton and on Chromodoris (cf. Arey and Crozier, '19; Crozier
and Arey, '19 b), with this result:

Blue. When Onchidia, in the dark, are made to crawl directly
toward the future source of light, and the blue light is then suddenly
turned on, the animals stop, pivot sharply through 180°, without
creeping, then move directly away from the light source. If started
in such a way as to creep in a direction perpendicular to the future light
source, the animals sometimes hesitate, lift the anterior end, and swing
it sharply away from the light source, thus making a precise 90° turn.

Green. When creeping is so begun as to lead the animal into the
light, a prompt turning through 180°, with subsequent locomotion
away from the light, at once follows the admission of the green light.
On the whole, the response is not quite so sharply carried out as with the
blue light. To unilateral light the response is as in the case of blue.

Yellow. To light impinging directly on the anterior end of the ani-
mal, Onchidium responds by creeping in a complete circle away from the
light, locomotion continuing along a path parallel to that at first pursued.
With unilateral light, the process of orientation is equally precise, but
less rapidly effected than in the case of green.

Red. In the majority of the trials, although Onchidium invariably
creeps away from the light, the orienting process is sometimes quite
slow, the animal occasionally continuing to move onward for several
millimeters after direct red light is turned on—then going off at a right
angle or turning back on its path. No cases were observed in which the
Onchidia pivoted immediately away from the light, as with the blue;
but on the contrary they maintained a steady creeping progress during
the course of orientation. When orientation is completed, the new
path does not quite coincide with the old, as in the case of blue or green
light, but in addition to being in the reverse direction lies within 10°
to 45° on one side or the other of the original course.

These experiments were made in a dark chamber, the only
source of light being a slit of appropriate size covered by the
appropriate ray-filter. The result of these tests, showing the
higher stimulating power of blue and green light, agrees with
what was found in the case of shading: under the blue or the
green ray-filter used in the phototropism tests, decrease of light
intensity led to a normal reaction on the part of the Onchidium,
while under the red or the green filter only a slight response, or

none whatever, could be secured; this was true even when the light falling on the blue filter was made very weak, while that admitted to the red filter was much stronger.

3. Thermal excitation

Under water Onchidium remains 'normal' in its behavior, so far as can be told, at temperatures between 17° and 36°C. If placed in sea-water cooled to 5°, the animal becomes instantly immobile, and does not respond to touch. After ten to fifteen minutes at this temperature, Onchidium quickly recovers if transferred to sea-water at the normal temperature (26° to 27°); in the early stages of this recovery no reactions of any sort could be elicited by tactile agents, but good reactions, involving contraction and curling up of the whole body, were elicited by the local application of small volumes of dilute HNO_3 ($N/200 \pm$). Sea-water cooled to 10° has practically the same effect. At 15°, an Onchidium suddenly placed in water of this temperature usually exhibits a few sluggish contractions of the foot musculature; if the animal is expanded, it contracts a little; if contracted, it relaxes somewhat and then remains quiet. Tactile irritability is present, but the responses are of slight amplitude.

Above 17°, probably, certainly above 20°, and until a temperature of 35° to 36° is used, no changes in the behavior of Onchidium indicative of sensory activation by heat are obtainable.

At 35° to 38°, Onchidia transferred to sea-water of these temperatures quickly become motionless, in the expanded state, except that if placed ventral surface uppermost there seems to be a slight but detectable increase in the peristaltic activity of the foot. They may make attempts to right themselves, but the bending movements involved in this process do not continue after two to three minutes.

With temperatures up to 45°, the result of immersing the animal suddenly is to call forth rather violent general contractions, more powerful the higher the temperature, but lasting less than one minute at 45°. Onchidium endures exposure to water of the latter temperature for about forty-five minutes; even after thirty minutes' exposure, it still reacts to touch and to stimulation by weak acid (HNO_3) solutions.

The lowest of the high temperatures which leads to death rapidly (i.e., within one minute) is very nearly 48°, although 47° is withstood for ten to fifteen minutes.

Local application of 'heat' or 'cold'—the tests being made by bringing a warmed or cooled glass rod into close proximity with a part of the snail, in air, or, since it was possible by careful manipulation to avoid tactile complications, bringing the glass rod directly into contact with the skin—provided no evidence of delicate thermal sensitivity. A rod cooled approximately to 0° C. called forth no detectable response whatever. A rod heated to 60°, or even 50°, did, however, cause prompt responses from all parts. The type of reaction was for each part of the body the same as in the case of touch, but more vigorously carried out. A light touch leading to no response at all calls forth a powerful reaction when administered with a warmed glass rod. The mimimal temperature effective in this way is about 45°.

In the case of an Onchidium detached from the substratum and resting on its back, the lower surface of the mantle may be touched repeatedly on one spot until the maximal amount of rolling-up occurs; there is then no further response obtainable to repeated mechanical stimulation, but a rod warmed to 45° (in reality, perhaps somewhat cooler at the moment of application) induces a sharp local contraction and drawing away of the mantle. Similarly, the back of an Onchidium may be repeatedly stimulated by pressing on it with a blunt point, by prodding or stroking it, until a response fails to be elicited; but a warmed rod, however lightly applied, calls forth a deep retractive puckering of the mantle over a relatively wide area.

The findings in these somewhat tedious experiments indicate the presence of a 'heat' sense physiologically distinct from that involved in touch, although (perhaps owing to the method of experimentation) the relative sensitivity of the various parts of the body seems the same as that already described for mechanical excitation. Sensitivity to 'cold,' on the other hand, is but poorly defined, if indeed it can be said to be present at all.

The resistance of Onchidium to high temperatures is decidedly greater than that of Chiton (Arey and Crozier, '19), which is worth pointing out because these animals live almost side by side, the Chitons probably being exposed to higher temperatures, and for longer periods, than Onchidium. The blue-black hue of most of the Onchidia may lead to the absorption of heat energy, however, during the intervals of creeping in the open. An adaptive basis might therefore conceivably be found for the relatively high heat resistance of the dark Onchidia used in these tests, if it should be found that these dark forms show a heat resistance superior to that of the pale variety obtained at nearby stations.

4. Chemical excitation

Onchidium does not react in any definitely detectable way when a small volume (0.5 cc.) of rain-water is applied to any part of its surface. Sea-water itself, so applied, may provide a mechanical stimulus unless the pipet current be very gentle; this effect, however, may readily be eliminated. Sugars (maltose, lactose, sucrose) in 1 M solution in rain-water were not found to induce responses. Sea-water concentrated to one-half its volume by evaporation and reaerated by bubbling air through it called forth contractile movements when applied to the head and lips; sea-water whose osmotic pressure had been increased by the addition of 0.5 M glycerin similarly induced slight reactions when applied to the lip region.

The osmotic disturbances which may induce responses about the mouth are much less effective as activating agents for Onchidium than are various chemicals, including alkaloids, anaesthetics, alcohols, acids, alkalies, salts, and such irritants as H_2O_2 and various essential oils. The whole surface of Onchidium is open to sensory activation through the agency of dilute solutions, such as those found effective for numerous other invertebrates (cf. Arey and Crozier, '19).

Small volumes of sea-water which had been shaken with ether, chloroform, carbon bisulphide, aniline oil, oils of thyme, origanum, juniper, bergamot, pennyroyal, cloves, or cassia, were

allowed to flow over a portion of the surface of Onchidium; the
reactions were in every case very vigorous, no matter what part
was immediately concerned in the activation—the foot, for
example, being found extremely sensitive.

Low concentrations of electrolytes, however, are more effec-
tive on the dorsal mantle and in the mouth region than on the
foot. The limiting concentrations beyond which the further
dilution of some representative materials no longer constitutes
sensory stimulants under the conditions of these tests—in a
general way similar to those found for other animals—are:

HCl—The foot ceases to react when the solution is more dilute than
M/300; the remainder of the body surface responds to dilutions as
great as M/500.
KOH—The limiting concentrations are about the same as for HCl,
but somewhat higher.
KCl—For the foot, M/3; for the rest of the body, roughly M/5 or
M/6.
Picric acid—At M/50 a weak response is still obtainable from the
foot; the limiting dilution for the general body surface is about M/3000;
at M/2000 the stimulation induced is still quite decided.

The animals were tested in air. The solutions were made up in
rain-water; with picric acid, solution in sea-water gave results
identical with those already tabulated.

It was attempted, for comparison with conditions in other
forms, to establish the relative stimulating efficiency of some of
the commoner ions. Solutions of the neutral salts of the alka-
lies, 5/8 M in rain-water, induced responses from all parts of
Onchidium. $MgCl_2$, even in 1 M solution, gave weak reactions
only, and none whatever upon the foot.

NaCl and LiCl led to the explosive discharge of the repugna-
torial glands, whereas KCl and NH_4Cl as a rule did not. When
local applications of the two latter salts were followed by a gland
discharge, it could be seen that the discharge was a secondary
phenomenon, due to the general squeezing of the glands through
the muscular contraction of the mantle, rather than to the
(normal) contraction of the investments of the glands them-
selves, the latter holding with NaCl and LiCl. NaCl in small
quantities led, on the whole, to more violent gland discharge

than did the same amount of LiCl. NaCl was more effective, also, on animals previously fatigued by repeated stimulation.

The foot is the part of Onchidium least responsive to ionic excitation; therefore it is in some respects a region advantageous for comparing the relative effectiveness of different substances. NaCl and LiCl induced pronounced puckering contractions when applied to any part of the foot, the animal being also induced to curl up, pill-bug fashion. KCl and NH_4Cl were very much milder in their effects; with KCl, tiny surface puckerings were induced, local wrinklings, but no deep local retractions such as were occasioned by NaCl; the efficiency of NH_4Cl was in this respect invariably less than that of KCl.

We arrive therefore at the following series, which expresses the relative efficiency of these cations at uniform concentration:

$$Na > Li > K > NH_4$$

The halides of potassium were used to obtain a corresponding series for the anions. KI and KCl were more effective than KNO_3 or KBr in stimulating the foot of Onchidium. While these two groups were sufficiently distinct, the further separation of the anion effects was a matter of some difficulty. The series eventually chosen as best expressing the results, in terms of the relative magnitudes of the reactions induced, was this:

$$I > Cl > NO_3 > Br$$

The cation series is practically the inverse of that usually found in such cases, and of that which we have found for the sensory activation of several other mollusks (Arey and Crozier, '19; Crozier and Arey, '19b), employing comparable criteria. The anion series is more like that commonly reported. The relatively great stimulating power of NaCl may be related to the fact that Onchidium creeps upon rock surfaces wet with sea-water, but exposed to the evaporating power of the sun. In this connection it should be noted that the mouth region is decidedly the most sensitive part, and also that in an actively creeping animal it is not possible, as a rule, to obtain sensory responses from activating substances at concentrations so dilute

as in the case of resting individuals. These features may play a part in directing the movements of Onchidium in nature.

There is some evidence for the view that receptors for general 'irritating' activation are distinct from chemoreceptors proper. Many substances which provide powerful sensory excitants for Onchidium do not induce discharge of the repugnatorial glands. Methyl or ethyl alcohol, however, at 5 M concentration in rainwater, do lead to such discharge; whereas pure amyl alcohol, directly applied, does not, although the general responses of the animal are in the case of the amyl alcohol much the more vigorous. Chloretone also, and urea, in relatively concentrated solutions, do not lead to discharge of the poison glands, although they do induce powerful reactions on the part of the animals as a whole.

An effort to effect physiological separation of tactile- and chemo-reception was without decisive result.

It may be noted that, according to Joyeux-Laffuie ('82, p. 238), O. celticum exhibits in its feeding a certain amount of preferential selection of Ulva from among a diversity of algae. He regarded this fact as evidence of a certain degree of 'gustatory' discrimination, associated with the mouth parts, but pointed out in addition that the oral lappets were employed in feeling over bits of algae before their being submitted to the radula. This latter observation we can confirm for O. floridanum, but we have seen no evidence of selective activity respecting food, perhaps because the thin algal carpeting of the rock in Onchidium habitats presents so uniform a field. Some of the older writers have referred to the 'eating' of silt by Onchidium; we have already pointed out (Crozier and Arey, '19 a) that shore-line silt is ingested, but in a purely fortuitous manner, through its adherence to the seaweeds.

ON THE ANALYSIS OF THE HOMING BEHAVIOR

An attempt to interpret or to reconstruct the natural activities of Onchidium on the basis of analytical study of its modes of response when removed from its habitual environment is confronted at once by some profound inconsistencies and by the

puzzle of 'homing' behavior. Although we have to offer some suggestions toward such an interpretation, they are presented with due reserve as to their probable finality. No more striking instance is known to us of apparent incompatibility between the results of controlled experimentation, repeatedly verified, and the most obvious activities of the same animal's natural life.

First, as to heliotropism. In the laboratory Onchidium behaves as a typical negatively heliotropic animal. In nature its behavior is in every essential at variance with this finding (Arey and Crozier, '18; Crozier and Arey, '19 c). Not only does this mollusk creep out of its dark nest into the glaring sun, when the tide is falling, to feed, but it does so only during daylight hours, and never at night, no matter how bright the moon. During the period of its emergence an Onchidium's movements seem not in any degree influenced by the sun. An individual quietly creeping in brilliant sunlight may be shaded by a board, and after re-expansion following its response to the shading, it creeps on as before. If now sunlight be thrown suddenly on this animal from a new direction, with a mirror, it may momentarily 'hesitate' somewhat, perhaps turn very slightly to one side, but soon it continues as before.

It is not merely the presence of a normal kind of shore substratum which determines this suppression of heliotropism, because animals brought from laboratory stock (secured several days before) and placed upon the same rock are oriented precisely by the sun. The presence of the specific rock surface normal to the Onchidia emanating from a given nest, namely, of that surface within a certain small radius of their nest entrance, is the deciding influence. An Onchidium moving toward its nest entrance, but heading directly into the sunset, may be picked up and then replaced upon the rock without interfering with its course. But if a glass plate be slipped under the animal before replacing it, it is now at the mercy of its heliotropism. If an Onchidium from one section of the shore be quickly transferred to a strange zone, again the animal is oriented by the light. Obviously, there is no question here of the mere rapid exhaustion of the heliotropic mechanism or other kind of 'light adaptation.'

It is not that the Onchidium, 'seeking its best interests,' comes out into the light to feed and in so doing ignores the dictates of its negative heliotropism. Definite evidence, on the contrary, is available to the effect that under normal circumstances the central nervous mechanism of heliotropic movements is inhibited, perhaps ordinarily by impulses originating in the oral lappets which compete successfully for the control of the body musculature. This inhibition can be abolished ('reversed') by means of strychnine (Crozier and Arey, '19c). Reversal of inhibition within the ganglia of mollusks is known in Chiton, in Chromodoris, and in Cephalopods (cf. Crozier, '20).

A possible explanation for the existence of negative heliotropism under any circumstances may be found in an imperfection of the photoreceptive system. The response of Onchidium to sudden shading is clearly of conceivable survival value; it leads to the retraction of the tentacles, the cessation of locomotion, and the depression of the mantle-fold to the substratum; the mantle is thus enabled to assist as a hold-fast, for the suction power of the foot is but poorly developed, and in any event the algal-covered surface affords a difficult field for contact attachment by the small foot of an animal so light in weight that it does not flatten out the algae. Our suggestion as to the nature of the heliotropic irritability, depending on the continuous chemical activity of incident light, involves the assumption that the stimulation so produced is in this case bound up with substances forming a necessary part of the receptive mechanism for the response to shading. The obviously efficient manner in which heliotropic impulses are normally blocked, in such fashion that they play no detectable part in controlling the creature's movements, may account for the fact that this deficiency in the photosensitizing mechanism has failed to suffer selective elimination. A balanced system of photocatalizable reactions was postulated to account for the phenomena of photic irritability in Holothuria (Crozier, '14). The general features of such a system have been (in Mya) admirably treated in a quantitative manner by Hecht ('19). For Holothuria it was suggested that both phases of activity within the photosensitive system—

photolysis in light of given intensity, and resynthesis in light of lower intensity—might be involved in stimulation by light and by sudden decrease of light intensity, respectively; here, both forms of irritability play rôles of bionomic importance (Crozier, '15), whereas, according to this notion, but one phase of the matter, namely, sensitivity to shading, is permitted to exert an influence upon the normal behavior of Onchidium. The mechanism whereby the possibility of heliotropic response is normally prevented has already been discussed (Crozier and Arey, '19 c). It appears to depend upon specific impulses originating in the oral lappets, at their points of contact with the substratum, for when these lappets are removed or are anaesthetized by $MgSO_4$, the Onchidium becomes photonegative, even if replaced upon the specific rock surface from which it was taken.

The rhythm of the tides, ordinarily well defined, controls the emergence of Onchidium upon its feeding ground. Under certain conditions the orderly succession of periods of low water is seriously interfered with. High winds and accompanying ocean currents, during times of storm, not infrequently cause such a 'piling up' of water within the semi-enclosed sounds at Bermuda that the water may fail to fall appreciably for several tides; such a period is followed, also, by a certain irregularity in the tidal sequence. Only when the water level has become lowered to the proper degree, previously indicated, do the snails emerge. Conversely, extensive periods of low water, notably occurring at spring tides, may leave the rocks uncovered along the Onchidium zone for an uncommonly lengthy interval. The duration of the emergence of a given colony is practically fixed, however, and gives not the least indication of being normally terminated by the rising of the tide; rather, the duration of the feeding time is determined in a quite different way, which we shall shortly consider.

The facts thus far presented do not exhaust the curious intricacies of the behavior of our snails. It has been mentioned that Onchidium comes out from its nest only during the daytime, and never at night. Were it not for certain serious obstacles, all this might be understood in terms of an hypothesis advanced

by Joyeux-Laffuie ('82), namely, that the tentacular eyes enabled the mollusk to distinguish between the darkened interior of its shelter and the illuminated outside feeding ground, thus guiding its emergence.[8] The difficulties facing such interpretation are: 1) the fact that the tentacles of O. floridanum, although there are tentacular eyes, seem non-photosensitive; 2) the fact that the creature is never positively heliotropic and, 3) the fact that emergence does not always occur when the tide is out during daylight hours. Good instances of the sort last mentioned were available in midsummer, when for several days at each lunar interval both tides were seen to expose the beach zone while the illumination was still quite good. At these times the snails were always found to emerge during but one tidal ebb, never during both. Once in each twenty-four hours is the maximum frequency of emergence. Even in the absence of conditions imposed by winds and currents impeding the escape of tidal water from the sounds, however, this rhythm does not represent the minimum frequency of emergence. For at neap tides the Onchidium nests highest above water may fail for some twenty-four hours to be submerged at flood tide, and in that case the Onchidia there located do not emerge until after their nest has been submerged. Moreover, in winter both morning and evening tides may fail for a day or so to occur during daylight hours; in this event, so far as we have studied the point (Dec., Feb., 1918), the Onchidia do not emerge at all until one tide occurs while the sun is up.

The diurnal rhythm thus clearly established 'in defiance of' the snail's heliotropism completely disappears in the laboratory. Fair-sized slabs of stone, a foot or so in breadth, were placed in aquaria containing freshly gathered groups of Onchidium. The animals always collected after a short time on the shaded, under side of such a slab, whether under water or in air. In the dark (artificial darkness or at night), they crept actively over the surface of their stones, feeding; the admission of light quickly caused typical photonegative retreat to the dark, under surface

[8] The fact that emergence occurs only during the daytime was not known to Joyeux-Laffuie.

of the rock. It was not possible, although several times the attempt was made, to establish an artificial tidal rhythm by periodically lowering the water level in such aquaria; this phase of the work will, we hope, be continued. Experiences of this sort plainly indicated the existence of some very specific correlation between the natural behavior of an Onchidium and the features of its ordinary home.

In our opening description of the chief phases in the daily life of O. floridanum we have already given a brief account of the most remarkable aspect of this specific correlation, namely, the snail's 'homing' behavior. It was obviously necessary to inquire into the nature of this peculiar activity. Although our results are not in any sense exhaustive, for we were unable to complete the series of experiments planned, the evidence we do command nevertheless permits a fairly precise characterization of the major aspects of the homing process. Involved in this matter are: 1) the fact of almost simultaneous commencement of the return to the 'nest' on the part of the scattered members of one colony, 2) the fact that the duration of the feeding interval seems automatically fixed without reference to the rising of the tide, and 3) the evidence concerning central inhibition, already referred to in connection with the normal abeyance of heliotropic movements. To these points we shall return, after dealing with the directed creeping toward the nest.

The fact that Onchidia are able to return to their nest after being picked up and replaced at some other point within a certain radius of the nest aperture is best appreciated from the perusal of such records as the following:

July 1, 1914. Little Agar's Island.
Five individuals were seen returning to a nest. Two of these were removed to the rock surface above the high-water mark (where, in our experience, these animals never wander naturally); the distance of the new point of departure was perhaps 40 cm. from the aperture of the nest. One of the displaced Onchidia turned directly toward the nest and crept straight back to it; the second one 'lost its way,' and wandered off in a strange direction. The three other members of the original group of five marched in a sort of triangular formation toward the nest aperture, two going to one side of the opening, the remaining one to the other side, then all three crept into the nest.

The nest was now broken into with a chisel. The three individuals inside were removed, and placed on a flat rock surface above the nest opening, and at three different points each some 46 to 50 cm. from the nest. All three Onchidia succeeded in effecting a return to the mouth of the nest. Near the nest two of them followed a slightly grooved trail; this trail or channel had also been used by the one returning individual described in the preceding paragraph. The channel led directly to the mouth of the nest. In getting into this trail, each individual had to change its course greatly. When they had reached the region about the nest aperture where the rock had been broken in examining the interior of the cavity, the Onchidia became much 'confused' and merely wandered about on the outside rock, where they were left as the tide rose.

July 2, 1914.

A group was noted returning to a nest, and one individual was picked up and replaced on the rock on the opposite side of the mouth of its nest at a distance of 1 meter therefrom. The animal returned directly to its nest.

In subsequent years many trials of this sort were carried out, and always with essentially the same result. It is possible, but not probable, that some interesting results would have been obtained by comparing carefully the homing capacity of Onchidia of different ages. In the autumn three groups of fairly distinct size are noticeable in the Onchidium population, so these snails probably live two years at least, if not more. Nevertheless, our experiments did not disclose any differences in homing ability among the individuals of different sizes. Factors which more noticeably affect the ability to 'home' after experimental displacement are the natural extent of the normal feeding area and the degree to which this area is populated with nests. These two factors are usually correlated quite closely. Boulder-like rocks more or less isolated from the shore are frequently so eroded as to present a veritable honeycombed aspect; a rock 3 feet by 2 in cross-section, projecting some two feet above m.l.w., was found to harbor about thirty Onchidium nests, if not more; less eroded rocks, often affording considerable expanses of flat surface, were seen to shelter an Onchidium population much less dense. In a habitat of the latter sort the distance limiting successful homing was about 1 meter, while experiments on rocks of the type first mentioned (on the south side of Dyer and of

Tucker's Island) showed that homing from distances greater than 30 to 40 cm. was not obtainable.

The fact that the course of an Onchidium when creeping out to feed may be quite 'haphazard,' zigzagging here and there, while the homeward course is usually as direct as the substratum allows, as well as the findings in experiments just cited, shows that it is not necessary for Onchidium to follow its own slime track. Limpets do adhere to their own tracks (cf. Davis, '95; Bethe, '98; Orton, '14, etc.); Bethe (loc. cit.) thought that limpets were guided in their return journeys by a sort of chemotaxis, which led them to follow their own slime trails. An Onchidium picked up when on its homeward journey and placed upon a clear glass plate in diffuse light does not tend to adhere to its own slime track, nor to the slime tracks of other individuals. The same result obtains with paper or the surface of a brick. Nor do these snails 'favor' a wet surface over a dry one (glass or filter-paper). An individual from a strange section of the shore put down on rock near an Onchidium nest will creep without hesitation across fresh trails of others.

All the facts which we have been able to gather about the homing of Onchidium may be brought into relation according to the hypothesis which we now set forth. Complete demonstration of the validity of this notion involves further experimentation, the nature of which we indicate.

The Onchidia in any one colony emerge from their nest after the tide has fallen so far as to have left it above water level for about a half hour to an hour. They scatter over the rock surface and feed. In the unfed condition certain sensory impulses otherwise directing and controlling the creature's movements in such fashion as to cause it to return to the nest are inhibited. The possibility of such central inhibition is given from the 'reversal of inhibition' with respect to phototropism seen under strychnine action while the snail is on the surface normal to it. After having fed for a certain time, substances derived from materials ingested while feeding pass into the juices of the snail's body and produce a 'reversal of inhibition' so far as the 'homing' impulses are concerned. Reversals of behavior follow-

ing feeding are known in such animals as the Porthesia cater-
pillars of Loeb ('18, p. 116), and the Planarians studied by Olm-
sted ('17 b). This hypothesis readily accounts for the fact that
the period of feeding lasts very nearly the same length of time
in all the members of a group.

The sensory impulses thus conceived to be released from
central inhibition through the results of feeding are regarded as
originating in the oral lappets. These well-developed 'cephalic
tentacles' are constantly in touch with the algal carpet of the
stone. If they are cut off, the Onchidium is 'lost,' unable to
return to its home. The removal of the dorsal tentacles, some-
times regarded as the seat of 'smell' in snails, has no such effect.

These impulses must be regarded as possessing the character-
istics of 'contact odors' (meaning thereby that perhaps both con-
tact and 'olfactory' stimuli of a certain kind must be received
simultaneously). The reason for this assumption is twofold:
in the first place, an Onchidium beneath which there is slipped a
glass plate is left thereby at the mercy of its heliotropism; sec-
ondly, an Onchidium will 'home' from points which it has not
previously visited; therefore, the aereal dissemination of some
guiding substance must be presumed. The olfactory com-
ponent of such a complex must be regarded as more important
than the tactile, for the rock surface above high-water mark is
not covered by algae as is the surface natural for Onchidium,
nevertheless the snails will 'home' from points on the former
surface, although in the ordinary course of events they never go
above high-water mark. They will not home when put under
sea-water, even if quite near their nest.

The substance providing a tropistic guide for a fed Onchidium
must be granted some highly specific quality. In view of
Bethe's findings for ant colonies ('98), such a supposition need
not be thought preposterous. Moreover, it is supported by some
striking results in our experiments on homing. In a number of
trials an Onchidium from one community was so placed that it
was forced to creep across the sunken gully leading to the opening
of a strange nest. Sometimes such a snail was found to follow
the new 'trail,' after a certain amount of preliminary turning

back and forth or 'hesitation,' and even to creep within the new entrance. But never, in these tests, did such a snail remain in the strange nest. Not infrequently several journeys were made about the foreign opening (Arey and Crozier, '18).

A further significant result was that in several of the tests made upon Onchidia taken from nests subsequently found to possess two apertures, the successfully homing snails gained entrance by way of a cleft different from that which they had followed in their undisturbed homeward trip.

The specificity of the assumed 'olfactory' substance is not 'remembered' by an Onchidium after twenty-four hours' confinement to the laboratory. This point was repeatedly tested at Dyer Island. Such confinement obliterates the possibility of homing to the old nest, even from distances of a few centimeters.

Our conception of the rôle of the oral lappets might be taken to explain the functional significance of certain curious glands located on these organs, in certain species. Plate ('94), with Oncis, and later Pelseneer ('01, p. 20), with Oncidiella patelloides, found on the sides of the oral lappets a pair of symmetrical apertures, the orifices of glands compared by Pelseneer to the anterior tentacular glands of Vaginula, but of unknown function. In O. floridanum, however, these glands are not present, otherwise one might suggest that these peculiar organs furnish a mucous covering for the oral lappets, perhaps containing a material serving as a specific solvent for hypothecated odoriferous emanations from the nest. It would be interesting to know how widespread the 'homing' may be among these related species.

This hypothesis not only accounts for all the facts known to us, as already stated, but obviously avoids reference to such obscurely defined notions as 'muscular memory' and the like. The hypothesis could best be tested by means of experiments upon the homing tendencies of Onchidia which had not been permitted to feed, and by the attempted discovery of the substance naturally responsible for our 'reversal of inhibition.' It should be noted that we distinctly avoid saying whether or not such substance may be derived from the algae ingested, because a certain amount of calcareous mud is also swallowed while feeding (Crozier and Arey, '19 a).

DISCUSSION

1. It is desirable to deal, as briefly as may be, with certain of the more general implications of the conclusions provided by our inquiry into the habits of Onchidium.

The Onchidiidæ are a group well calculated to cause the zoölogist trouble. For a long time the taxonomic affinities of these organisms were hazy and in dispute, for it was by some (Bergh, '95; Fujita, '97) supposed that, in addition to dermal respiration accomplished through mantle papillae (conspicuously developed in certain species) when under water, air breathing was also carried out, but by the organ regarded as a kidney—an idea once used as the foundation of von Ihring's class 'Nephropneusten,' but now known to have resulted from an imperfect acquaintance with the difficult morphology of the true lung (Plate, '94; von Wissel, '98; Pelseneer, '01). Thus we are probably dealing with a member of a typical land group, Pulmonata, which has secondarily taken up the habit of living on the seashore. It would be valuable to know whether Onchidella is a more archaic type than Onchidium proper (Plate, '94), or the reverse. According to Bretnall ('19), Onchidium dämelii lives either altogether below low water or between tidal limits.

2. The activities of these animals are not less curious than their presumptive evolutionary history. In the case of numerous invertebrates of the shore zone it has seemed possible to provide a clear description of behavior in terms directly stated by the outcome of analytical experiments. In fact, this general method of study has been made the basis of much recently published work in animal ecology. The ethology of Chiton (Arey and Crozier, '19) and of Chromodoris (Crozier and Arey, '19b) can be followed with gratifying completeness from relatively simple experimental results. With Onchidium the situation is more subtly complicated, and for the purpose of ecologic interpretation the isolated results of such a method are here almost meaningless, as shown conspicuously by the creature's heliotropic responses. Michael ('16) and others have recently been to some trouble to emphasize the fairly obvious point that no

amount of mere laboratory investigation makes it absolutely certain that we may predict the movements of an animal in nature. In reality, however, it is largely a question of the relative completeness with which experimentation is conducted; nor does it require much penetration to discover that the necessary degree of completeness may differ in various cases.

A point of some interest, although perhaps unduly speculative, concerns the historical source of Onchidium's heliotropic machinery on the receptor side. That the possibility of heliotropic orientation does not entrain adaptive consequences, seems adequately demonstrated by a previous discussion (Crozier and Arey, '19 c). But most land pulmonates are negatively heliotropic. Might it then be conceived that the sensory organs involved in this form of irritability are mere 'holdovers' from the more ancient stock? Aside from the fact that the skin of at least some snails and slugs is photosensitive (Yung, '10), very little information useful in this connection has been discovered. It must be remarked that the mechanisms for sensitivity to light and to shading are seemingly closely connected, if not identical, in Onchidium; nothing of this sort is known for other pulmonates. More important, however, is the conclusion of Stantschinsky ('07) regarding the origin of the dorsal eyes (mantle-eyes) in the family of Onchidia: he has shown it probable, on general morphological grounds, that the more highly developed forms of mantle photoreceptors are indeed primary, rather than a secondary development, and that species, therefore, such as O. floridanum, which lack the dorsal eyes have arrived at this condition through secondary degeneration.

Yung ('13) holds that certain gastropods are 'blind,' their tentacular eyes being non-functional, and that this is due to the fact that the optic nerve fibers fail to penetrate the basement membrane of the retina. The lack of apparent functional activity in connection with the tentacle eyes of O. floridanum might be of interest in relation to this conception, were it not for the fact that the conditions here may not involve a complete absence of innervation of the retinae. So far as they have been made out from carefully studied sections, the relations of the

'optic' nerve in Onchidium seem to be as follows: The tentacular nerve, entering at the base of a tentacle, runs mainly to the periphery of the tentacle, ramifies there, and ends in intimate association with numerous large, clustered nerve cells near the tip of the tentacle; at the level of the eye-cup, a small ramus is split off from the main course of the nerve and passes to the eye, but an actual connection with it, such as is easily seen in many molluscan eyes, is exceeding difficult to demonstrate; our evidence seems to show, however, that a few fibers perhaps do actually enter the optic cup. This structural state may be indicative of degeneration.

If the mantle receptors of O. floridanum must be regarded as mere remnants of the original photosensitive equipment of this stock, the possibility of their connection with a primitive heliotropic mechanism in ancestral pulmonates acquires an unprofitable vagueness. We have thought it necessary to raise this point because it has sometimes been held that non-adaptive responses "have been inherited from ancestors in which they were adaptive" (meaning that the mechanism for response has been so inherited). For Onchidium such interpretation is highly improbable.

3. Neither can the heliotropism of Onchidium be dismissed as a mere 'laboratory product.' Some writers have endeavored to account for heliotropic orientations as found in various animals on the basis that determinate movements of this character must be the result of 'abnormal' conditions (cf. Franz, '13). It will be obvious that notions of this sort cannot affect the analysis of the mechanism of photic orientation, but can refer only to the rôle of heliotropism as an ethologic factor. It is only in a very limited sense that the heliotropism of Onchidium may be regarded as 'unnatural.' It is not that laboratory conditions artificially imposed determine the orientations so produced, but on the contrary that in surroundings other than the immediate environment of the 'home' nest some specific factor producing central nervous inhibition of what may, for convenience, be termed the (sensory) heliotropic impulses, fails to appear. It is sufficient to remember that an Onchidium need only be transferred to a new

section of the shore in order to witness the complete unmasking of its heliotropic impulses.

Since strychnine has in some instances been shown to produce negative phototropism, even in animals naturally indifferent to light (Moore, '12), it should be clearly understood that the strychnine effect in Onchidium cannot be regarded as of this sort.[9]

Certain animals are known to become photonegative upon immersion in sea-water. Isopods of the genus Ligia, which in certain places occupy territory also frequented by Onchidium, have been said by Abbott ('18) to reverse their phototropism, perhaps under control of humidity, in the sense that at low tide they come out from hiding places above flood-tide level and wander over the exposed intertidal zone. That this behavior really involves phototropism of any kind, and is not rather a case similar in certain features to that of O. floridanum, remains to be proved. It would be of interest to test this matter, for Abbott states that "in the laboratory they (Ligia) give a negative reaction to sunlight." Moreover, an understanding of the situation in Onchidium may be important for the elucidation of other curious cases in which an animal's heliotropism seems fundamentally at variance with its mode of life (e.g., Paravortex, described by Ball, '16, p. 464).

According to Mitsukuri ('01), the specific habitat of Littorina is determined by changes in its phototropism, from negative in air and under water to positive when splashed by waves. Here, again, the evidence that phototropism is really primarily involved is somewhat defective. The heliotropism of Onchidium is in no respect altered by complete immersion in sea-water.

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[9] Whether the action of strychnine in producing negative heliotropism with an animal naturally photopositive or even indifferent to light (Moore, '12, '13) can be always referred to chemical modifications within the primary receptors, rather than to some more strictly central nervous (synaptic) effect, remains unanswered. Even with the human eye, where visual acuity (retinal resolving power) is notably augmented by strychnine, one cannot at present be sure that the removal of certain central inhibitions is not at bottom responsible. As an example of the inhibition of one sensory impulse by another, we might cite the heightened tactile responsiveness of certain de-eyed fishes (Crozier, '18).

For reasons already amply set forth, we must reject the notion that the movements of Onchidium involve, or depend upon, any 'reversal' of phototropism. From the standpoint of adaptation, the heliotropic mechanism must be regarded as a most interesting example of a perfectly definite functional characteristic which proceeds automatically from the given physicochemical composition of the organism (cf. Loeb, '16), without reference to adaptive requirements (cf. Arey and Crozier, '18; Crozier and Arey, '19 c). Since the young of Onchidium (developing within the nest) emerge from the egg capsule with the form of the adult, and not as veligers (Joyeux-Laffuie, '82), and since we have found very tiny individuals (2 mm. long) emerging from nests with adults, it cannot be said that perhaps at an early stage these animals are normally photonegative, by this means first becoming established in their definitive nest.

4. A number of instances are on record of the preservation in the rhythmic activities of animals of some diurnal or tidal rhythmicity inherent in the environment (Wilson, '00; Gamble and Keeble, '00; Schleip, '10; Keeble, '10; Esterly, '17; Cary, in Dahlgren, '16, reprint p. 11, etc.). Unfortunately, a number of such reports, especially those concerning the persistence of environmental rhythms in actinians, have proved to be the result of erroneous observation (Parker, '19). We were interested to discover if, in a form like Onchidium exhibiting such complex responses, there would be found any persistence of either tidal or nycthemeral rhythms of activity and repose, in the absence of the rhythmic excitations normally associated. It can be said with confidence that no rhythms of this character are maintained by Onchidium when removed to the laboratory.

5. For some time it has been known that the limpets and their allies inhabiting the tidal zone may at times wander for some little distance from, and subsequently return to, the 'scar' indicating their definite 'home.' The literature of this subject has been reviewed in an interesting way by Piéron ('09 c). A certain complication enters here, for some limpets creep forth from their scar when covered by the sea, others only when left bare by the tide. Piéron (loc. cit.) has given a plausible account

of these differences, though certain of his statements could be better weighted with evidence.

The general fact of the wandering of limpets from their scars has been known since the time of Aristotle, and the fact of homing has more recently attracted the notice of a number of naturalists (Bethe, '98; Davis and Fleure, '03; Piéron, '09 a, '09 b, '09 c, '19; Bohn, '09; Orton, '14; Billiard, '14; Wells, '17). It is all the more curious that so little close experimental work has been devoted to the elucidation of the matter. Homing activities are shown by a number of more or less distinctly related forms— Patella, Siphonaria, Helicon, Fissurella, Calyptraea; among these, various degrees of 'homing' ability are manifest, and in Acmaea testudinalis, according to Willcox ('05 b), there is no evidence of this activity at all. 'Homing' is found to be successfully executed by these animals even when they are artificially removed from their scar or from some point along their feeding path and replaced within a reasonable distance of the scar. For Patella, Piéron ('09 c) records successful returns following a displacement of 12 cm., while, in certain localities, the extent of the natural feeding journeys may be as great as 55 to 90 cm., according to various observers quoted by Piéron. For other genera less distances limit the mollusc's successful return to its home subsequent to experimental shifting—with Siphonaria, 2 to 3 inches or perhaps a little more; for Fissurella, 2 inches (Willcox, '05 a).

This kind of 'homing' has certain resemblances to that of Onchidium, yet there are noteworthy differences. Piéron notes that some individuals (Patella) wander little or not at all in securing food; these are easily 'lost,' and do not succeed in returning to their scars if even slightly displaced. Piéron regards the homing as dependent upon a permanent memory of the topography of the habitual situation, and upon a very exact memory of the relief of the spot on which the Patella orients itself according to the irregularities of its shell.[10] He succeeded in demonstrating that a Patella could so orient itself,

[10] Cf. Piéron, '19.

even when the margin of the shell had been chipped away, and concluded therefore that the 'topographic memory' involved must be a sensory affair. The deduction is reasonable that the cephalic tentacles are the essential guiding organs, particularly in creeping, and that the pallial tentacles serve this function while the Patella is adjusting its irregularly outlined shell to the depression of the scar. But it should not be forgotten that the experimental test of this conclusion, particularly in so far as it pertains to the somewhat obscure 'topographic memory,' has yet to be instituted.

With reference to the bearing of these findings upon the analysis of 'homing' in Onchidium, we need only point out that the homeward creeping of the latter has a much more flexible cast than is true of the behavior of the limpets, especially in those experiments where an Onchidium removed from one entrance of its nest and replaced upon the rock was found to gain the nest again, but by a second, different aperture. The distances from which a successful return is effected are also notably greater in the case of Onchidium. Nevertheless, the probability of any deep-seated 'memory' of the location of the nest is negatived by the fact that confinement to the laboratory for twenty-four hours obliterates the capacity for return to the nest. Tests of this point with Fissurella and Patella are of great interest; according to Piéron ('09 b), the ability of Patella to return to a particular 'home' can survive some days' removal from the scene. Even bees are said to lose their memory of specific locations after being anaesthetised (quoted from Minnich, '19).

The behavior of limpets is of greater significance in connection with the possible evolution of the 'homing' capacity. Something of this sort seems to have been in Wells's ('17) mind. It can be pointed out that several grades of increasing precision and complexity of 'homing' activity are shown by molluscs (Arey and Crozier, '18). Beginning with Chiton tuberculatus (Crozier, '21), in which there can be found something like 'homing,' but of a rather vague type and pretty certainly the result of immediate stimulations, a series comprising also Patella,

Onchidium, and Octopus exhibits more and more highly developed 'homing' propensities. The return of a Patella, Fissurella, Siphonaria, or Calyptraea to its specific site cannot be accomplished beyond a relatively slight distance; these creatures also tend to follow fairly definite paths in their excursions and to adhere to these paths when returning; and some of them creep but slightly, if at all, away from their scars. Onchidium's behavior is obviously an advance in respect to complexity. Analogous behavior has been described for snails and slugs (as in the famous story of the sick snail and its companion, cited by Darwin, '71, p. 316, and by others; cf. also Cooke, '95, and Scharff, '07). The investigation of this matter in snails and slugs holds the possibility of considerable interest. Finally, the behavior of Octopus (cf., e.g., Cowdry, '11), which returns to its nest after extensive forays and from considerable distances, under circumstances such that direct vision of the nest entrance is completely excluded, represents the most complex form of this activity among molluscs.

There has been a tendency to regard any series of this kind as exhibiting stages in the evolution of a particular response, or even of an instinct. To speak of a 'homing instinct' is little short of a perversion of sense. Such a view-point is very likely quite incorrect. Much more probable is it that this series of forms displays merely stages in the evolution of the central nervous machinery making possible more and more complicated behavior. The phrase 'evolution of an instinct' tends to obscure the real basis of the matter. Moreover, in the special instance under discussion, it is not at all obvious that the actual 'homing' performances of the several types named are in any sense genetically connected; any relation with the mechanism of homing in higher forms, birds, for example, is in the highest degree improbable. Even the homing of ants involves certain characteristics, such as those described by Cornetz ('14), which are not in any sense represented in the behavior of Onchidium.

We early recognized the simulation of associative memory in the activities of Onchidium (Arey and Crozier, '18) with reference to its nest. If the notion of such memory or 'beginnings of

intelligence' be valid for cephalopods (v. Uexküll, '01; Polimanti, '10), it is legitimate to inquire if anything of this nature can be imputed to Onchidium. According to Miss Thompson ('17), the snail Physagyrina, although in maze experiments it gives no evidence of learning, does exhibit the establishment of simple associations when tested by Pavloff's method of 'conditioned responses.' There is no real evidence favoring the idea of memory as evinced in the 'homing' of O. floridanum. One adequate test of the conceivable action of associations or even of primitive intelligence has occurred to us. When an Onchidium is picked up and put down on a strange portion of the shore, it cannot, of course, find its old nest; but other nests and various unoccupied crannies are available for shelter. The fact is, however, that instead of seeking the shelter afforded by 'strange' crevices, the snail is on the contrary at the mercy of two chief modes of response: its negative phototropism and its withdrawing reaction when shaded; that specific quality of its own particular nest which probably determines homing makes it possible for the creature to enter its own nest notwithstanding its photic sensitivities. Strayed Onchidia do not find shelter in new cavities of the rock, but on the contrary creep about on the shore until covered and washed off by the returning tide. Evidence of intelligence or of adaptive use of associative memory is completely absent, although, as we have elsewhere remarked (Arey and Crozier, '18), the close simulation of behavior of that order is certainly deceptive.

SUMMARY

Onchidium (Onchidella) floridanum is a small naked pulmonate inhabiting the intertidal shore zone at Bermuda. The individuals of this species are grouped together into colonies numbering about a dozen individuals, more or less, in each. A colony during high water occupies a 'nest,' in the form of an eroded cavity in the shore rock or a cleft between clay-cemented stones. During the day-time only, and at most but once in the twenty-four hours, the Onchidia emerge from their nest after the falling tide has left it above water for about an hour. The animals feed for a fixed period of about one hour, then those

individuals emanating from a given nest begin simultaneously to execute a direct return to the nest from which they originated. These animals will not enter a 'foreign' nest.

When tested apart from their specific normal environment the Onchidia are always negatively phototrophic. In the natural state their movements are entirely independent of heliotropism. This independence can be obliterated by injected strychnine, which produces 'reversal of inhibition.' Similarly, the simultaneous return to the nest on the part of the various members of a colony can be understood on the assumption of a 'reversal of inhibition' brought about by substances derived from materials ingested while feeding.

The impulses which, on this hypothesis, suffer central inhibition in the outwardly creeping snail may be identified with those which normally control the determinate character of the homeward course. These impulses probably originate in the oral lappets, and are taken to have the character of a 'contact odor' (see text) specific for each particular nest.

This is the only hypothesis which can account for the observed peculiarities of the movements of Onchidium and for the outcome of the experiments concerning homing reported in this paper.

There is no evidence of associative or persisting memory in connection with homing, nor do other activities of Onchidium point to the existence in this form of anything approaching intelligent behavior. Responses to immediate stimulations are adequate for the analysis of the situation.

The negative heliotropism of Onchidium, apparently devoid of adaptive significance, is accounted for in terms of a photosensitive receptor system enabling these snails to respond to shading by an effective use of the mantle as a hold-fast, supplementing the weak suctional efficiency of the foot. The existence of receptors making negative heliotropism possible cannot be understood as a condition persisting from ancestral pulmonates normally responding in this way.

Mantle eyes are absent in this species, and although the tentacular eyes are perhaps of normal structure, no photic sensitivity has been discovered in connection with them.

The snails do not emerge to feed when high winds and surf are directed against their feeding zones. The tentacles are anemotropic organs, which prevent the emergence of the Onchidia in such circumstances.

An analysis has been given of some of the snail's modes of response, and of certain general implications of the remarkable activities of Onchidium.

LITERATURE CITED

ABBOTT, C. H. 1918 Reactions of land isopods to light. Jour. Exp. Zoöl., vol. 27, pp. 193–246.

AREY, L. B. 1918 The multiple sensory activities of the so-called rhinophore of nudibranchs. Amer. Jour. Physiol., vol. 46, pp. 526–532.

AREY, L. B., AND CROZIER, W. J. 1918 The 'homing habits' of the pulmonate mollusk Onchidium. Proc. Nat. Acad. Sci., vol. 4, pp. 319–321.
  1919 The sensory responses of Chiton. Jour. Exp. Zoöl., vol. 29, pp. 157–260.

BALL, S. 1916 The development of Paravortex gemellipara (Graffilla gemellipara Linton). Jour. Morph., vol. 27, pp. 453–557.

BANCROFT, F. W. 1913 Heliotropism, differential sensibility, and galvanotropism in Euglena. Jour. Exp. Zoöl., vol. 15, pp. 383–428.

BERGH, R. 1895 Über die Verwandtschaftsbeziehungen der Onchidien. Morph. Jahrb., Bd. 10, S. 172–181.

BETHE, A. 1898 Dürfen wir den Ameisen und Bienen psychische Qualitäten zuschreiben? Arch. f. ges. Physiol., Bd. 70, p. 15–100.

BILLIARD, J. 1914 Sur la locomotion chez les patelles. Bull. Soc. Zool. France, no. 7, pp. 325–326.

BOHN, G. 1909 De l'orientation chez les patelles. C. R. Acad. Sci., T. 148, pp. 868–870.

BRETNALL, R. W. 1919 Onchidiidæ from Australia and the Southwestern Pacific Islands. Rec. Austral. Mus., vol. 12, pp. 303–328.

BROWN, T. G. 1914 On the nature of the fundamental activity of the nervous centers; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. Jour. Physiol., vol. 48, pp. 18–46.

CHAMBERLAIN, R. V. 1920 The myriopod fauna of the Bermuda islands. Ann. Ent. Soc. Amer., vol. 13, pp. 271–302.

COOKE, A. H. 1895 Molluscs, in Cambr. Nat. Hist., vol. 3, pp. 1–459.

CORNETZ, V. 1914 Les explorations et les voyages des Fourmis. Paris.

COWDRY, E. V. 1911 The color changes of Octopus vulgaris Lmk. Contrib. Bermuda Biol. Sta., no. 22 (vol. 2); from: Univ. Toronto Stud., Biol. Ser., no. 10, 153 pp.

CROZIER, W. J. 1914 The orientation of a holothurian by light. Amer. Jour. Physiol., vol. 36, pp. 8–20.
  1915 The sensory reactions of Holothuria surinamensis Ludw. Zool. Jahrb., Abt., Physiol., Bd. 35, S. 233–297.

CROZIER, W. J. 1917 The nature of the conical bodies on the mantle of certain nudibranchs. Nautilus, vol. 30, pp. 103–106.

    1918 On tactile responses of the de-eyed hamlet (Epinephelus striatus). Jour. Comp. Neur., vol. 29, pp. 163–173.

    1919 a On the use of the foot in some mollusks. Jour. Exp. Zoöl., vol. 27, pp. 359–366.

    1919 b On the resistance of Fundulus to concentrated sea-water. Amer. Nat., vol. 53, pp. 180–185.

    1919 c On the control of the response to shading in the branchiae of Chromodoris. Jour. Gen. Physiol., vol. 1, pp. 585–591.

    1920 On the analysis of neuromuscular mechanisms in Chiton. *Ibid.*, vol. 2, pp. 627–634.

    1921 The question of 'homing' behavior in Chiton. Amer. Nat. (In press.)

CROZIER, W. J., AND AREY, L. B. 1918 On the significance of the reaction to shading in Chiton. Amer. Jour. Physiol., vol. 46, pp. 487–492.

    1919 a Onchidium and the question of adaptive coloration. Amer. Nat., vol. 53, pp. 415–430.

    1919 b Sensory reactions of Chromodoris zebra. Jour. Exp. Zoöl., vol. 29, pp. 261–310.

    1919 c The heliotropism of Onchidium: a problem in the analysis of animal conduct. Jour. Gen. Physiol., vol. 2, pp. 107–112.

DAHLGREN, U. 1916 The production of light by animals (Porifera and Coelenterata). Jour. Franklin Inst., Feb., pp. 1–19.

DALL, W. H., AND SIMPSON, C. T. 1901 The mollusca of Porto Rico. U. S. Fish. Comm. Bull. for 1900, vol. 1, pp. 351–524.

DARWIN, C. 1871 The descent of man, vol. 1. New York.

DAVIS, J. R. A. 1895 The habits of limpets. Nature, vol. 51, pp. 511, 512.

DAVIS, J. R. A., AND FLEURE, H. J. 1903 Patella. Liverpool M. B. C. Mem., X. London.

DOLLEY, W. J., JR. 1917 The rate of locomotion in Vanessa antiopa in intermittent light and in continuous light of different illuminations, and its bearing on the 'continuous action theory' of orientation. Jour. Exp. Zoöl., vol. 23, pp. 507–518.

ELIOT, C. 1899 Notes on tectibranchs and naked mollusks from Samoa. Proc. Acad. Nat. Sci. Phila., 1899, pp. 512–523.

ESTERLY, C. O. 1917 The occurrence of a rhythm in the geotropism of two species of plankton copepods when certain recurring external conditions are absent. Univ. Calif. Publ., Zoöl., vol. 16, pp. 393–400.

FRANZ, V. 1913 Die phototaktischen Erscheinungen im Tierreiche und ihre Rolle in Frei-leben der Tiere. Zool. Jahrb., Abt. Physiol., Bd. 33, S. 259–286.

FUJITA, T. 1897 [On the method of respiration in Onchidium.] (Japanese.) Dobutsu Gaku Zasshi (Zoöl. Magaz.), vol. 7.

GAMBLE, F. W., AND KEEBLE, F. W. 1900 Hippolyte varians: a study in color-change. Quart. Jour. Micr. Sci., N. S., vol. 49, pp. 589–699.

GARREY, W. 1918 Light and the muscle tonus of insects. The heliotropic mechanism. Jour. Gen. Physiol., vol. 1, pp. 101–126.

GEE, W. 1913 The behavior of leeches, with special reference to its modifiability. Univ. Calif. Publ., Zoöl., vol. 11, pp. 197–305.

HECHT, S. 1919 Sensory equilibrium and dark adaptation in Mya arenaria. Jour. Gen. Physiol., vol. 1, pp. 545–558.

HESS, C. 1912 Gesichtssinn, 3: Akkommodation. In: Winterstein, Handb. vergl. Physiol., Bd. 4, 9, S. 709–840.

HIRASAKA, K. 1912 [On the structure of the dorsal eye of Onchidium.] (Japanese.) Dobutsu Gaku Zasshi (Zool. Magaz.), vol. 24, pp. 20–35.

JENNINGS, H. S. 1907 Behavior of the starfish, Asterias forreri de Loriol. Univ. Calif. Publ., Zoöl., vol. 4, pp. 53–185.

JOYEUX-LAFFUIE, J. 1882 Organisation et développement de l'Oncidie. Arch. Zool. expér. et gén., T. 10, pp. 225–383.

KAFKA, G. 1914 Einführung in die Tierpsychologie, Bd. 1: Die Sinne der Wirbellosen. Leipzig.

KEEBLE, F. 1910 Plant-animals: a study in symbiosis. Cambridge.

LOEB, J. 1916 The organism as a whole. New York.
1918 Forced movements, tropisms, and animal conduct. Philadelphia.

MICHAEL, E. L. 1916 Dependence of marine biology upon hydrography and necessity of quantitative biological research. Univ. Calif. Publ., Zoöl., vol. 15, pp. i–xxiii.

MINNICH, D. E. 1919 The photic reactions of the honey-bee, Apis mellifera L. Jour. Exp. Zoöl., vol. 29, pp. 343–425.

MITSUKURI, K. 1901 Negative phototaxis and other properties of Littorina as factors in determining its habitat. Annot. Zool. Japon., vol. 4, pp. 1–19.

MOORE, A. R. 1912 Negative phototropism in Diaptomus by means of strychnine. Univ. Calif. Publ., Physiol., vol. 4, pp. 185–186.
1913 The negative phototropism of Diaptomus through the agency of caffein, strychnine and atropin. Science, N. S., vol. 38, pp. 131–133.

MURRAY, J. 1885 Narr. of the Chall. Expedition, vol. I, pt. 1 (Onchidium, p. 489).

OLMSTED, J. M. D. 1917 a Notes on the locomotion of certain Bermudian mollusks. Jour. Exp. Zoöl., vol. 24, pp. 223–236.
1917 b Geotropism in Planaria maculata. Jour. Anim. Behav., vol. 7, pp. 81–86.

ORTON, J. H. 1914 Note on the feeding habits of Patella. Jour. Mar. Biol. Assn., N. S., vol. 10, pp. 254–257.

PARKER, G. H. 1911 The mechanism of locomotion in gastropods. Jour. Morph., vol. 22, pp. 155–170.
1919 The elementary nervous system. Philadelphia.

PATTEN, B. M. 1915 A device for projecting a small spot of light suitable for exploring photosensitive areas. Science, N. S., vol. 41, pp. 141–142.

PELSENEER, P. 1901 Études sur les gastropodes pulmonés. Mem. Acad. Sc. Roy. Belg., T. 54.

PERRIER, E. 1917 Sur les échanges de faune entre la mer et les eaux donces et les conséquences qu'ils entraînent au point de vue de la sexualite. C. R. Acad. Sci., T. 165, pp. 748–751.

PEYRÉGA, E., ET VLES, F. 1913 Notes sur quelques relations numériques relatives aux ondes pédieuses des Gastéropodes. Bull. Soc. Zool., Fr., 1913, pp. 151–254.

Piéron, H. 1909 a Sens de l'orientation et mémoire topographique de la Patelle. C. R. Acad. Sci., T. 148, pp. 530–532.
1909 b Contribution à la biologie de la patelle et de la Calyptrée. Le sens de rétour et la mémoire topographique. Arch. Zool. expér. et gen., Notes et Rev., ser. 5, T. 1, pp. xviii–xxix.
1909 c Contribution à la biologie de la patelle et de la calyptrée. Bull. Sci. Fr. et Belg., T. 43, pp. 183–202.
1919 De l'importance respective des divers facteurs sensoriels dans le sens du retour de la Patelle. C. R. Soc. Biol., T. 82, pp. 1227–1230.

Plate, L. 1894 Studien über opisthopneumone Lungenschnecken, II. Zool. Jahrb., Abt. Anat., Bd. 7, S. 93–234.

Polimanti, O. 1910 Les céphalopodes ont-ils une mémoire? Arch. de Psychol., T. 10, pp. 84–87.

Ribaut, H. 1915 Biospeologica: xxxvi. Notostigmorpha, Scolopendromorpha, Geophilomorpha. Arch. Zool. expér. et gén., T. 55, pp. 323–346.

Romanes, G. J. (1912, repr.) Animal intelligence. New York.

Scharff, R. F. 1907 European animals. New York. 14 + 258 pp.

Schleip, W. 1910 Der Farbenwechsel von Dixippus morosus (Phasmidae). Zool. Jahrb., Abt. Zool., Bd. 30, pp. 45–132.

Semper, K. 1877 a Reisen im Archipel der Philippinen, Theil 2, Bd. 3, Landmollusken, Ergänzungsheft, S. 1–46, Taf. A–E.

Semper, C. 1877 b Ueber Schneckenaugen vom Wirbelthiertypus nebst Bemerkungen über einige andere histologische Eigenthumlichkeiten verschiedener Cephalophoren. Arch. mikr. Anat., Bd. 14, pp. 117–124.
1881 Animal life as affected by the natural conditions of existence. New York.

Sherrington, C. S. 1906 The integrative action of the nervous system. New York.

Stantschinsky, W. 1907 Zur Anatomie und Systematik der Gattung Oncidium. Zool. Jahrb., Abt. f. Syst., Bd. 25, p. 353–402.
1908 Über den Bau der Rückenaugen und der Histologie der Rückenregion der Oncidium. Zeit. wiss. Zool. Bd. 90, S. 137–178.

Thompson, Elizabeth L. 1917 Experiments with Physagyrina. Behav. Monogr., Cambr., iii, no. 3, p. 1–89.

Uexküll, J. von. 1909 Umwelt und Innenwelt Tiere. Berlin.

Wells, M. M. 1917 The behavior of limpets, with particular reference to the homing instinct. Jour. Anim. Behav., vol. 7, pp. 387–395.

Willcox, M. A. 1905 a Homing of Fissurella and Siphonaria. Science, N. S., vol. 22, pp. 90, 91.
1905 b Biology of Acmaea testudinalis Müller. Amer. Nat., vol. 39, pp. 325–333.

Wilson, C. B. 1900 The habits and early development of Cerebratulus lacteus (Verrill). A contribution to physiological morphology. Quart. Jour. Micr. Sci., N. S., vol. 43, pp. 97–198.

Wissel, K. von 1898 Beiträge zur Anatomie der Gattung Oncidiella. Zool. Jahrb., Suppl. 4 (Fauna Chilensis, Bd. 1), S. 583–640.

Yung, E. 1910 La sensibilité des gastropodes terrestres pour la lumière. Arch. Sci. Phys. et Nat., T. 30, pp. 617–618.
1913 La cécité des gastéropodes pulmonés. Arch. Sci. Phys. et Nat., T. 35, p. 77.

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