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BY

WESLEY R. COE

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INTRODUCTION

The season of attachment, rate of growth, and age at sexual maturity of the native oyster on the coast of southern California have been recently determined (Coe, '32) by means of a series of experimental blocks set in the water at intervals throughout the year and examined at stated periods. Through the cooperation of Professor W. E. Allen, of the Scripps Institution of Oceanography, data are now available for each of the five years 1926 to 1931.

In connection with this work microscopic sections of the gonads have been made at all ages up to the third and fourth sexual phases. The gonads of older animals have also been studied from other localities on the California coast. For this latter material the writer is indebted to Dr. G. E. MacGinitie, of the Hopkins Marine Laboratory at Pacific Grove.

It has long been known that *O. lurida,* like the European *O. edulis,* is hermaphroditic and viviparous, but little information was available as to its sexual phases until Stafford ('13) published his report on the "Canadian oyster." Stafford observed that on the coast of British Columbia the younger individuals produced only sperm and rightly concluded that the species is protandric. Examination of the gonads of older animals showed that some parts of the reproductive system might contain ova while in other gonads in the same individual only spermatozoa were present. He came to the decision that the young are male at all seasons, but that older individuals are male at the beginning of the reproductive season and female at its height, the sperm ripening and being discharged before ovulation.

In the investigations recorded in the present paper it was found that the sexual conditions of this species at La Jolla are somewhat at variance with Stafford's conclusions, for there appears to be a regular rhythmical sequence of sexual phases, the type of sexuality in which an individual begins each breeding season, after the first, depending upon the phase which it had reached in the preceding autumn.

Orton ('27) has made a thorough study of the sexual phases of *O. edulis,* supplementing the previous work of Hoek ('83), Spärck ('25), and others. Orton produces evidence to show that this European species is also protandric, with female and male phases alternating regularly, following the initial male phase. The factors determining the sequence are hereditary rather than environmental, although the surrounding influences may control the rate at which the alternating sexual phases proceed. This seems to be equally true of the California oyster.

In this connection it should be noted that the spawning season of the oyster extends over a period of at least seven months on the coast of southern California, the first spat usually appearing on the experimental blocks in April, while the last spawning of the year occurs in October or November (Coe, '30).

Although the mature oyster may contain ripe sexual products during the winter, these are not discharged until the temperature of the water reaches at least 16° C.[1] Ovulation in the autumn is cor-

[1] In average years this critical temperature is reached during April, but in 1931 the mean temperature of the surface water for the first two weeks in February was 16.2° C. This followed unusually warm water during January. Consequently spawning commenced this year fully two months earlier than usual, as was proved by the attachment of spat on the experimental blocks. The abnormally high temperature, with accompanied spawning, continued through March and April.

respondingly inhibited when the temperature approaches this critical point. Galtsoff ('30) has found for *O. virginica* and *O. gigas* that the spawning stimulus involves both a suitable temperature and the presence of spermatozoa in the water.

Perhaps it should be added that in *O. lurida,* as in the European oyster, the eggs are fertilized within the mantle cavity of the parent, where they remain and develop until the larvae have reached the bivalve condition. Shortly after they have been spawned into the water these young bivales attach themselves to almost any kind of solid objects. The free-swimming stage is thus very short and the opportunities for dispersal are limited. *Ostrea equestris,* found occasionally on the Atlantic coast of the United States, has been shown by Gutsell ('26) to have similar habits.

DEVELOPMENT OF PRIMARY GONADS

The reproductive system, when fully developed, lies in the reticular connective tissue close beneath the surface epithelium and extends over almost the entire body. An irregular branching and anastomosing system of genital canals on each side of the body—upper and lower, or right and left—leads to the pair of genital pores which open just ventral to the adductor muscle.

When the gonads first appear, the layer of connective tissue between the surface epithelium and the internal organs—stomach, intestine, digestive gland, excretory organ, heart—is very thin, but as the gonads increase in size the layer of connective tissue becomes correspondingly thicker (fig. 1).

The branching canals form the superficial portions of the primary gonads, the sex cells developing on the opposite, or inner, sides. A series of evaginations of the inner walls of the primary gonads leads to the formation of the tubular secondary gonads which penetrate the underlying connective tissue in a direction vertical to the surface of the body. The genital canals retain their positions close beneath the surface epithelium and eventually become lined with cubical ciliated cells (fig. 1), as Hoek ('83) and Spärck ('25) have described for *O. edulis.*

The first indication of the cells which are to form the gonads is found at an age of about eight weeks after attachment during the warmer seasons of the year, but at a somewhat later age in those individuals in which the gonad first develops in the winter. Many connected groups of only three or four cells each, situated in rows in the connective tissue close beneath the epithelium of the body, constitute the fundaments of the reproductive system.

At the age of twelve weeks some of the primary gonads still consist of only a few cells (fig. 2, *a*), while in others the cells have increased in number and the gonads have already acquired lumens. These flattened, anastomosing chambers now extend over almost the entire surface of the body. The germinal epithelium soon becomes differentiated into two types of cells, primary ovogonia and primary spermatogonia, with a few smaller, undifferentiated epithelial cells which later become incorporated into the lining of the genital canal.

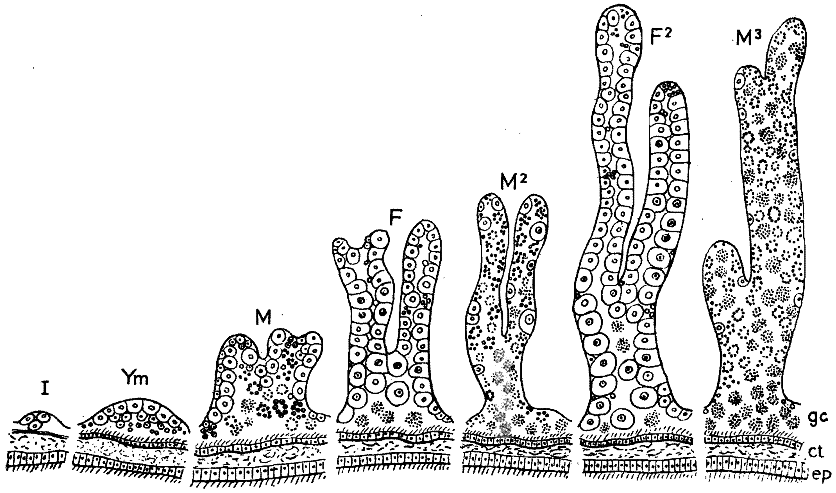

Fig. 1. Diagram showing the development of the gonads, their position relative to the body wall and the sequence of sexual phases. *I*, indifferent juvenile phase; *Ym*, primary gonad of young male, with ovogonia and spermatogonia; *M*, male, first phase, spermatogenesis with evaginating secondary gonad containing ovocytes; *F*, female, first phase, with clusters of spermatozoa remaining in genital canal; *M2*, male, second phase; spermatogenesis; a few ovocytes remain; *F2*, female, second phase; secondary gonad larger; a few sperm-balls remain; *M3*, male, third phase, spermatogenesis; few ovocytes on walls of follicle; *ep*, ciliated epithelium of body wall; *gc*, genital canal, with ciliated outer wall; *ct*, connective tissue.

The ovogonia are distinguished by their larger nuclei (fig. 2, *b*, *c*) with conspicuous nucleoli and loose chromatin network; they usually lie in contact with the wall of the gonad. The spermatogonia have much smaller nuclei, with more condensed chromatin and are frequently separated from the wall of the gonad by the larger ovogonia. Both types of germinal cells occupy the inner side of the lumen (fig. 2), while the cells of the later genital canal lie on the side nearest the surface of the body.

At the age of about twenty-two weeks the gonads have increased greatly in size and their lumens are sometimes almost obliterated by the rapidly proliferating germinal cells (fig. 2, *e*). The ovogonia and ovocytes still line the inner wall, with a few undifferentiated cells

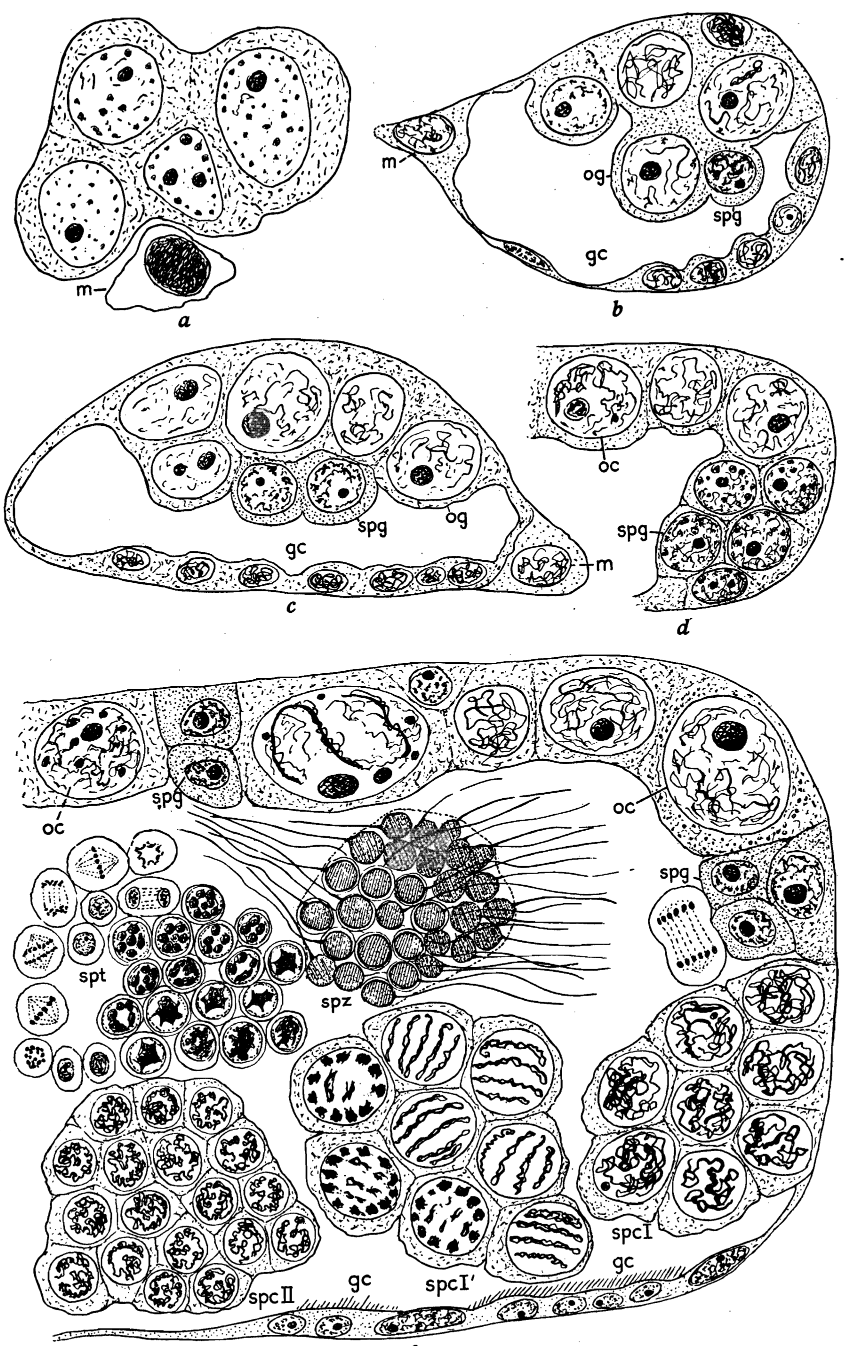

Fig. 2. Development of primary gonad. *a,* Primitive indifferent gonad with four cells and accompanying mesenchyme cell (*m*); *b,* cavity of gonad forming, with ovogonia, and spermatogonia; *c,* lumen complete, with canal cells below; *d,* multiplication of spermatogonia (*spg*) and differentiation of ovocytes (*oc*); *e,* primary gonad, young male phase, with spermatogenesis in progress; *spc I,* primary spermatocytes, with chromatin in leptotene, synapsis, and prophase; *spc II,* secondary spermatocytes; *spt,* spermatids; *spz,* small sperm-ball; *gc,* genital canal; *oc,* ovocytes; *og,* ovogonia. *a-d,* 12 weeks, and *e,* 22 weeks, after attachment of spat.

apparently destined to remain inactive until they later form the spermatogonia of the second male phase. The spermatogonia proliferate much faster than do the ovogonia and give the early gonad its male characteristics. Spermatogenesis is soon in progress and a few small clusters of spermatozoa are formed. Most of the lumen, however, is occupied by groups of spermatogonia and spermatocytes in active growth and division (fig. 2, *e*). Not infrequently all the nuclei of a large cluster of spermatogonia or of primary or secondary spermatocytes will be found in process of mitosis.

With the completion of spermatogenesis the genital ducts are crowded with clusters, or balls, of spermatozoa. In each ball the hundreds of spermatozoa derived from a single primary spermatogonium are firmly imbedded. The sperm are then ready to be discharged from the body (pl. 7, figs. 5, 6).

SEQUENCE OF SEXUAL PHASES

Although the first male phase is followed by a regular alternation of female and male phases throughout the life of the individual (fig. 1), the lower temperature during the winter may delay the completion of any one of these phases for several months, so that a phase may be inaugurated in October or November and completed in April. The same phase might be completed within a few weeks in the summer.

The sequence is further complicated by the fact that not all parts of the reproductive system reach any particular phase of sexuality at exactly the same time. There is, in fact, great variability in this respect, for in young animals a single section through the body sometimes shows stages of gametogenesis that would be interpreted as quite different sexual phases if their true relations were unknown (fig. 4). In some cases sections through anterior and posterior ends of the body would certainly have been thought to come from different individuals. Furthermore, the genital canals, particularly of young individuals, in the typical female phase usually contain more or less numerous sperm balls remaining from the previous male phase, while the ovarian follicles usually have spermatogonia anticipatory of the following male phase (pl. 7, figs. 5, 6). For these reasons the experimental studies that have been made by Spärck ('25) and others to test the sequence of phases in *O. edulis* through small holes bored in the shells may not be entirely reliable. More than a single small sample of the gonad is often necessary to determine the condition of the entire reproductive system. But in older individuals the sequence of phases occurs over the entire body with greater precision.

In addition, all possible intergradations between the different phases of the gonads are found in young animals, so that it is frequently impossible to assign the individual to any one of the principal phases of sexuality. In the transition stages from female to male, free ova may be present in the same lumens that contain ripe spermatozoa in addition to spermatocytes and spermatids (pl. 8, figs. 9, 10). Both sorts of gametes may then be found together in the genital canals, so that there can be little doubt that in many cases both ova and spermatozoa may be discharged into the mantle cavity at the same time.

The fact that this frequently happens, however, does not prove that self-fertilization will result, for the sperm balls hold the spermatozoa firmly until they are liberated by contact with the sea water. Nor is it known whether the two sorts of gametes from one individual are capable of mutual reaction even if mingled in the sea water. It has been stated that ova of *O. edulis* at the moment of ovulation may have been already fertilized by sperm, possibly derived from other individuals, which have collected in the ducts through which the eggs pass. More recent observations, however, indicate that such is not ordinarily the case, and that segmentation does not usually begin until an hour or more after ovulation (Orton, '27).

The number of sexual phases which an animal passes through in one year is probably variable, depending on nutritive and other conditions. During its first year at least three phases commonly occur if the young is hatched in early spring. The initial male phase and the succeeding female and male phases may be completed before the end of November, when ovulation and sperm shedding are checked over the entire colony. In other animals the second male phase may be continued until the following April. Orton ('27) concluded that in *O. edulis* four sexual phases were sometimes completed in one season on the coast of England.

The following table indicates the condition of the reproductive system as shown by a study of microscopic sections of oysters of known ages. In order to condense the data as much as possible the following abbreviations are used: *I,* indifferent juvenile phase; *Ym,* young male in process of differentiation; ***M,*** first male phase; ***M2,*** and ***M3,*** second and third male phases; ***Mf,*** first male phase passing into female; ***M2f,*** second male phase passing into female; ***F,*** first female phase; ***F2,*** second female phase; ***Fm,*** first female phase with some retained ova, passing into second male phase.

In some cases different parts of the system show enough variability to warrant classification in two categories, as indicated. The ages given represent the total time that the blocks were in the water. The actual ages will always be somewhat less, and in some cases much less, owing to the time which may have elapsed between setting the blocks and the attachment of the young. The conspicuously smaller, and presumably younger animals on each block are therefore not included.

Besides the sixty individuals recorded in Section 1 of this table, serial sections of the gonads of more than one hundred additional animals of approximately known ages have been studied. Although different seasons of the year and different age groups are represented, all are in conformity with the data included in the table.

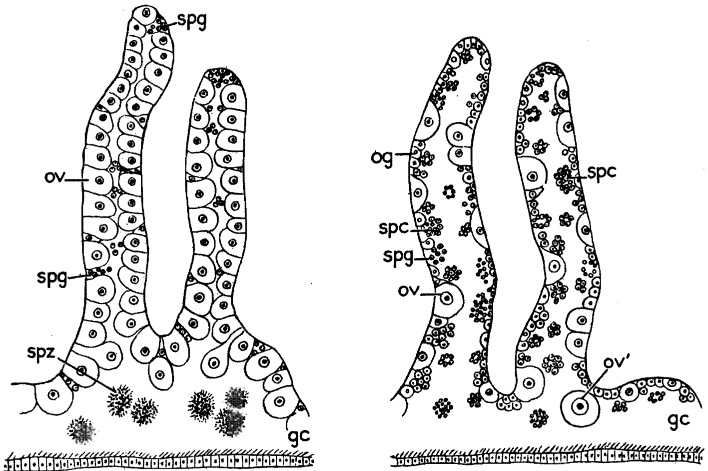

Fig. 3. Comparison of gonads before and after ovulation. *a*, climax of female phase; ovarian follicles with closely packed ova (*ov*), interspersed with small groups of spermatogonia (*spg*) which supply the germinal cells for the succeeding male phase; a few sperm-balls (*spz*) from the preceding male phase remain in the genital canal (*gc*). *b*, Gonad soon after ovulation (from animal with segmenting eggs in mantle cavity); early male phase with proliferating spermatogonia (*spg*) and spermatocytes (*spc*); a few large ova (*ov*) remain, one of which (*ov'*) is free in genital canal.

Inspection of the table will show that while the primary gonads have become differentiated and spermatogenesis is beginning within twelve weeks after the attachment of the spat in midsummer, about twice that length of time is required to reach the same stage during the cooler portions of the year. The shell at this stage has reached a length of 12–15 mm. in the warm season, or about 20 mm. in the cooler periods.

If the temperature of the water is suitable when the climax of the first male phase occurs, the ripe sperm balls will then be dicharged. At this time the ovocytes begin to grow rapidly to form the follicles of the secondary gonad, as the male phase is gradually succeeded by the female (fig. 3).

At the age of about twenty-two to thirty weeks most of the sperm balls have been shed, the ovarian follicles have formed and the first ova are ready for liberation. This female phase may be retained for several weeks, accompanied by two or more periods of ovulation, each of which is followed by the spawning of the young. One case was observed where a second ovulation had occurred before all the young of the earlier brood had been spawned, for eggs in the early stages of segmentation and a few black bivalved young were found together in the mantle cavity.

Some spermatogonia always remain after most of the ripe ova have left the follicles of the secondary gonad and these then begin a rapid proliferation. This is followed by the successive stages of spermatogenesis, and within eight to twelve days after ovulation, or about the time of the spawning of the young, the second male phase is reached (pl. 8, figs. 9, 10). Following the ripening and discharge of the sperm balls a period of recuperation often ensues, and this is followed by the second female phase. After the second ovulation period the animal functions as a male for the third time, followed by another resting period (fig. 1). This rhythmical sequence presumably continues through the remainder of the animal's life (Coe, '31*a*).

Various modifications of these sexual phases, partly due to the different seasons when the young are hatched, remain to be discussed.

MODIFICATIONS OF THE TYPICAL SEXUAL SEQUENCE

Since ovulation may occur at any time from early April (February in 1931) to October or November there will be great variation in the age of the different individuals at the time of the first ovulation. Instead of at the age of twenty-two to twenty-six weeks, as in summer, the first ovulation may not take place until the individual is at least eight months old if it was hatched late in the autumn. Two generations within twelve months are thus theoretically possible, but it is unlikely that such are of frequent occurrence even in the most favorable situations on the coast of southern California, except in such years as 1931, when the spawning temperature was reached two months earlier than usual. One generation and a partial second is presumably the general rule (Coe, '30).

Although a regular alternating sexual rhythm is typical, it has been mentioned that all the ripe eggs may not be discharged at one time and a second ovulation may follow immediately after one crop of larvae has been liberated. If a temperature of below 16° C intervenes between these successive ovulations it seems not improbable that one of the spawnings may take place in the autumn and the subsequent one the following spring (pl. 8, fig. 10). Nor is it inconceivable that one sexual phase or the other may be greatly reduced in the later years of the animal's life. Such individuals would approach the dioecious condition. The tendency toward maleness or femaleness in associated animals of the same age is sometimes quite pronounced.

Moreover, it may eventually be found that the populations of different regions vary in this respect, as is known to be the case in some other pelecypods, notably *Anodonta,* where Weisensee ('16) found the populations living in streams to be dioecious, while those individuals of the same species inhabiting quiet ponds are hermaphroditic.

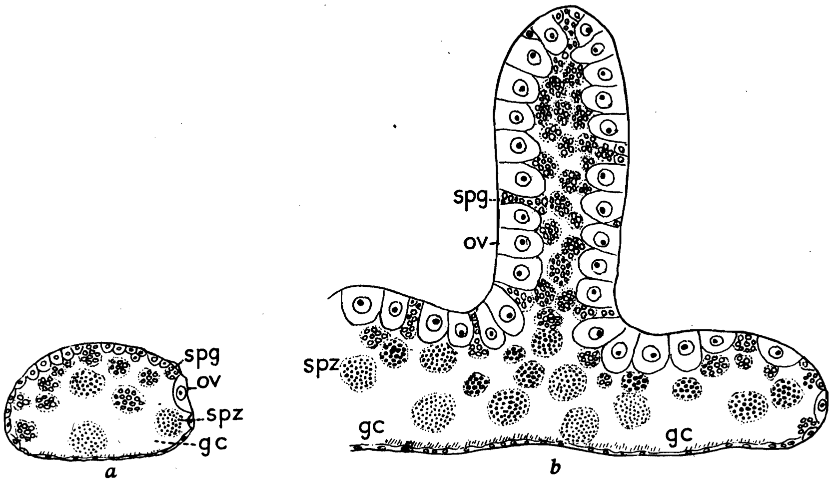

Fig. 4. Two gonads, drawn to same scale, from a single microscopic section of young male twenty-eight weeks after attachment (Aug. 4 to Feb. 16), showing great difference in development. *a,* primary gonad with few minute ova; *b,* ovarian tubule forming secondary gonad, with large ova and with spermatogenesis in lumen; *gc,* genital canal; *ov,* ova; *spg,* spermatogonia; *spz,* spermatozoa.

The age at the first spawning presumably also varies in different localities, for in *O. edulis* the first crop of young may be discharged in the animal's first year of life on the coast of southern France but not until its third or fourth year on some parts of the Danish coast (Spärck, '25).

TRUE HERMAPHRODITISM

In some cases fully ripe ova may lie free in the follicles and canals in which spermatogenesis is in full progress (pl. 8, fig. 10). Ova and sperm balls mingle freely in the genital canals and are doubtless discharged simultaneously into the mantle cavity. The solvent action of the sea water in this cavity where the eggs are retained presumably liberates the spermatozoa from the balls in which they are imbedded, offering abundant opportunity for self-fertilization. Likewise, sections of the gonads of ripe females (fig. 3) almost always show at least a few sperm balls remaining from the previous male phase. These had evidently been delayed in ripening until after the main crop of sperm balls had been discharged. The great variability which occurs in regard to the relative proportions of male and female elements in different follicles in a single animal is indicated in figure 4.

TYPICAL PROTANDRY

If the young become attached late in the season and have a long period of growth before discharging their first crop of gametes the following spring, the gonads will have become much more voluminous than they are in those individuals of the spring broods which spawn late in summer. In the former case the gonads are packed close together and each follicle has a thick wall of ovogonia and ovocytes, with proliferating groups of male cells filling up the lumen. The preliminary male phase reaches its climax in time for the ripe spermatozoa to be discharged as soon as the water reaches the critical temperature of 16° C, usually early in April. The very abundant female gametes will then ripen immediately. The second male phase soon follows.

Individuals which become attached to the blocks in July or early August may complete the initial male phase and shed their sperm late in autumn. The first female phase then develops during the winter in preparation for ovulation in early spring. Other animals on the same blocks, but not necessarily of the same age, reach only the first male phase during the winter and the genital canals become packed with sperm balls. When the temperature becomes suitable in early spring sperm-shedding by these individuals will accompany ovulation by their neighbors.

SPERMATOGENESIS

As stated above, the spermatozoa always leave the body grouped into sperm balls (spermatophores). These are spherical clusters usually composed of from 250 to upwards of 2,000 ripe gametes. Since all the spermatozoa in a ball originate from a single primary spermatogonium, this and its descendants must divide six to nine times to form a spherical group of from 64 to 500 primary spermatocytes. Each of the latter, after passing through typical synaptic and growth stages divides twice in the usual manner to form the spermatids (Coe, 31*b*).

Each spermatid takes a radial position in the spherical group, with its growing flagellum extending outward. The ripe spermatozoa are thus oriented with all the heads toward the center of the ball and the tails extending radially in all directions (figs. 2, 3, 5). They thus pass through the genital ducts into the body cavity and are then expelled from the body by the spasmodic contractions of the shell. On reaching the sea-water the sperm balls rotate rapidly, due to the lashing of the multitudinous sperm tails; in contact with the sea-water the balls gradually disintegrate and the individual spermatozoa are liberated to swim about independently in the same manner as other flagellated sperm (Coe, '31*b*).

SECONDARY GONAD—DEVELOPMENT OF FEMALE PHASE

While spermatogenesis is in progress in the primary gonad, and before the spermatozoa have been discharged, the ovogonia on the inner sides of the lumens engage in a rapid proliferation, forming ovarian invaginations into the parenchyma adjacent to the digestive gland. The lumens are first densely packed with spermatogonia and later with sperm balls (fig. 2, *c*). After the discharge of most of the sperm the ovarian invaginations continue their growth as slender tubules of rapidly growing ovocytes (pl. 7, fig. 6). Small clusters of indifferent gonia and small spermatogonia are carried along with the ovocytes to supply the germinal cells of the second male phase and such additional sexual phases as may occur during the remainder of the animal's life.

Occasionally the secondary follicles are composed mainly of spermatogenic cells, the growth of many of all of the young ovocytes being inhibited until spermatogenesis is nearly completed, giving rise to "secondary males" and "true males."

GONADS IMMEDIATELY AFTER OVULATION

The ripening and discharge of the ova apparently stimulate the spermatogonia which have been slowly proliferating in the ovarian tubules, for active spermatogenesis immediately follows. Plate 8, figures 8 and 9 indicate the changes that may take place during the period between ovulation and spawning. A series of preparations shows that there is a fairly close correlation between the time elapsed after ovulation and the stage of spermatogenesis in the gonads, as has been already reported for *O. edulis*. Of 702 individuals of that species which Orton ('27) found carrying young in the mantle cavity, about 50 per cent of the individuals showed only early stages of spermatogenesis in the first few hours after ovulation, while those carrying shelled larvae had an abundance of ripe sperm balls. The development of sperm was found to continue for about a month after ovulation. Two to three months after ovulation the male phase begins to wane and a small percentage of individuals immediately assume the female phase again. Usually, however, about twelve months intervene between two female phases on the coast of England. In cooler regions this phase is assumed only in alternate years, with a similar periodicity in maleness, while in unusually cold summers spawning may be omitted for two or more successive years (Spärck, '25).

SECOND MALE PHASE

If ovulation has been complete or nearly complete, the reproductive system quickly assumes the male phase, with spermatogenesis in full activity while the embryos are being carried. The few remaining ripe ova may eventually undergo degenerative changes and phagocytosis. But if the developing embryos in the mantle cavity represent only a portion of the ripe ova, the gonads may still be of predominatingly female type with some of the follicles lined with large ova, while clusters of spermatogonia fill up the lumens. Follicles with fewer large ova have correspondingly more spermatocytes, with some spermatids and a few spermatozoa. The follicles from which the ova in the mantle cavity were liberated can sometimes be identified by their large, nearly empty lumens with only a few spermatogonia and spermatocytes (pl. 8, fig. 8).

The secondary gonads are now so much more extensive than were the primary gonads of the first male phase that the numbers of sperm balls produced is vastly greater and a larger proportion of them bear the maximum number of spermatozoa (fig. 1).

RESTING PERIOD

During the growth of the young oyster its soft, flabby and translucent body has gradually become firmer and of a whitish color as the gonads of the first male phase increase in size. During the shedding of the sperm there is but little loss in volume, for the first female phase follows immediately thereafter. Following ovulation there is some decrease in the plumpness of the body but the whitish color is retained during the rapidly ensuing male phase.

With the discharge of the spermatozoa at the end of this second male phase, however, there frequently comes a marked change in the appearance of the body, for the tissues which hitherto have been plump, firm and whitish now become flabby, watery and translucent. A period of recuperation with a retardation in the formation of gametes usually ensues. But if the nutritional conditions are favorable the recuperation period may be abbreviated or eliminated and the second female phase will quickly follow. In such cases there may be several changes of the sexual phase in a single breeding season.

SECOND FEMALE PHASE

During the resting period the secondary gonads still further increase in size, both by an addition to the number of follicles and by the further branching and lengthening of such follicles as were present in the second male phase (fig. 1). As a general rule the animal is now considerably larger than at the first female phase and the number of ova produced is much greater (pl. 7, fig. 6; pl. 9, figs. 11, 12). The shell and body have greatly increased in size, so that a much larger number of embryos can be accommodated in the mantle cavity and the brood at spawning is correspondingly more numerous.

THIRD MALE PHASE AND LATER SEXUAL PHASES

Immediately after the second period of ovulation, the gonads assume the male phase again, but most of these gonads differ from those of the first two male phases in having few, if any ovocytes and only a few small ovogonia. The animal is now recognized as a ''true'' male instead of a partial hermaphrodite as in the preceding male phases. The number of sperm balls produced is enormously greater, due to the greatly increased size of the system (fig. 1; pl. 9, fig. 11).

In a similar manner the later female phases show a reduced intersexuality, the large ovarian tubules having relatively few spermatogonia until after ovulation (pl. 9, fig. 12).

WINTERING CONDITION

There seems to be no marked tendency for any particular sexual phase to appear at any definite period of the year. All phases are found in an unselected lot of oysters both in summer and in winter. Sections were made of the gonads of thirty-four mature individuals (see table 1, section 2) collected in February and therefore shortly preceding the discharge of any of the sexual products formed during the winter. Nearly half of the total number, or fifteen (Nos. 61–75) of the thirty-four, were typical intersexual forms or individuals showing the transitions from one phase to the next. Five (Nos. 76–80) were predominantly males, with the gonads and ducts enormously distended with sperm balls; four (Nos. 81–84) were almost exclusively ripe males, with minute ovogonia only; six (Nos. 87–92) were ripe females with a few sperm balls in the genital canals; one (No. 93) was female with many sperm balls; one (No. 94) was female with the canals distended by sperm balls (pl. 7, fig. 7); one (No. 86) was female with spermatogonia, but no later stages of spermatogenesis (pl. 9, fig. 12), and one (No. 85) was truly hermaphroditic, with both ova and sperm balls free in the lumens and canals (pl. 8, fig. 10).

Inspection of table 1, section 2, will show that all except one of the thirty-four individuals contained at least some ripe sperm-balls and that in some of these the bodies were greatly distended with sperm. The presence of sperm, on superficial examination, might lead to the conclusion that most of the individuals were truly males, but this was not the case, for twenty-five of the thirty-four had well-grown ovocytes also.

It seems evident that in the population from which these samples were taken there would occur a wide-spread emission of sperm shortly after the critical temperature was reached in the spring. Judging only by the appearance of the gonads, this would be accompanied by ovulation on the part of about one-fourth the total number of animals. But experience shows that the gonads may appear to be ripe a long time before ovulation actually takes place. Ovulation by about two-thirds the remaining population would follow as soon as the M2f and M3f individuals had reached the climax of the female phase. All the others would function as females later in the season. Only a very small percentage of the population liberate their ova in the early spring, for the height of the spawning season is not reached until June or July (Coe, '32).

Individuals of known age show that there is a continuation during the winter of the phase which the animal had reached in the autumn, accompanied by a great increase in the size of the gonads. If a particular phase was almost completed in the autumn there may be a degeneration and phagocytosis of at least part of the remaining sexual cells of that phase and a proliferation of the cells of the succeeding phase. A change of phase occurs during the winter only when the inhibition period in the autumn coincides with a completed or nearly completed sexual phase.

If selection is made on the basis of size, the majority of the largest individuals are in the female phase during the winter, presumably indicating a tendency for the older animals to pass through a single long female phase, followed by a completed male phase each breeding season. The smallest are invariably hermaphroditic males.

HERMAPHRODITISM IN OTHER SPECIES

Hermaphroditism is not uncommon in the genus *Ostrea,* at least ten of the more than sixty described species being known to be monoecious and viviparous. Several of the oviparous species show at least a small percentage of intersexual individuals, especially when young, and one well-known species of commercial importance has been recently shown to be protandric (Roughly, '28, '29). Furthermore, Burkenroad ('31) has now presented evidence that even the common commercial oyster (*O. virginica*) is protandric on the coast of Louisiana, with an incidence of about one per cent of hermaphroditism in the total population. I have also found a considerable percentage of intersexuality in small individuals of the same species at Woods Hole, Massachusetts and at New Haven, Connecticut.

A most interesting type of sex change has been recently reported by Amemiya ('29) for the Japanese oyster (*O. gigas*), previously considered dioecious. In one summer a small hole was made in the shell of each of several hundred oysters and the sex thereby determined. The sexes were then placed in separate cages and returned to the sea. A year later it was found that oysters of both sexes were present in each cage. It was concluded that about 25 per cent of the females and 60 per cent of the males had changed their sex during the winter. If these conclusions prove to be well founded, Amemiya's hypothesis, that the sex of any individual of that species is determined each winter independently of its previous sexual conditions, will add a new phase so the many variants of sexuality in animals.

GENETICAL EXPLANATION

Since the evidence seems conclusive that the initial male phase in all individuals of *O. lurida* is followed by a rhythmical sequence of alternating female and male phases throughout life, but with great variations in the predominance of one sex phase or the other, it may be assumed that all the eggs are alike as regards their primary sexual potencies and that the sexual phases are controlled by supplementary hereditary units which come into action in a rhythmical sequence. It seems not improbable that thse genetic factors may be themselves primarily controlled by an hereditary rhythm in metabolism, as Orton ('27) suggests. It also seems quite possible that later investigations may show that environmental conditions such as, perhaps, the close association of younger individuals with older, sexually mature animals may have some influence on the sexual phases of the former, as is well-known to be the case with some other mollusks, and as the evidence presented by Burkenroad ('31) indicates in *O. virginica*.

Homozygosity of the primary sex factors in *O. lurida* may be correlated with opportunities for self-fertilization.

SUMMARY

1. On the coast of southern California the native oyster produces spawn during at least seven months of the year, but spawning is inhibited when the temperature falls to about 16° C.

2. Spawning is resumed when the temperature of the water exceeds 16° C for some days in succession. In the early spring most of the mature animals are provided with at least some ripe sperm, but only about 25 per cent of them have ripe ova.

3. The height of the spawning season occurs in June and July.

4. Functional gametes may be formed at an age of from 20–30 weeks in summer; two generations a year are theoretically possible, but probably only one and a partial second usually occur.

5. All individuals are protandric and hermaphroditic, with a rhythmical sequence of alternating female and male phases following the initial male phase.

6. All phases of sexuality are found in an oyster colony at all seasons of the year due to the overlapping of broods.

7. The primary gonads form as small associations of germinal cells in the connective tissue immediately beneath the body walls; a lumen soon appears in each gonad, the cells on the side nearest the body wall forming the ciliated epithelium of the genital canal, with which the gonad is continuous, while those on the opposite side of the lumen become differentiated into ovogonia and spermatogonia.

8. The spermatogonia proliferate rapidly to form the cells of the first male phase, but before the sperm are mature the ovogonia have produced numerous ovocytes, giving the gonad its intersexual character.

9. Coincident with the later stages of spermatogenesis the proliferation of ovogonia and the growth of the resulting ovocytes leads to the formation of the follicles of the secondary gonads of the first female phase as tubular invaginations into the underlying connective tissue in a direction vertical to the surface of the body.

10. The second male phase follows immediately after ovulation. A period of recuperation frequently precedes the second female phase.

11. The sexual phases in the young animals intergrade to such an extent that more than 90 per cent of the population are obviously

intersexual. In microscopic sections from 238 individuals no exclusively male or female animal was found. Even older animals in the female phases usually retain sperm balls in some parts of the reproductive system, as well as small spermatogonia in the gonads; in the male phases large and small ovocytes are usually present in addition to the small ovogonia. In some old animals transition stages may be greatly reduced.

12. Opportunity for self-fertilization is frequently present, for both ova and sperm balls may be discharged simultaneously into the mantle cavity.

13. There is no evidence that season, temperature, or other environmental conditions can alter the inherent rhythmical sequence of the sexual phases, other than to prolong or shorten any of the phases and decrease or increase the number and proportion of gametes produced. From a genetical standpoint it seems probable that the sex-differentiating factors are comparable with similar rhythmical hereditary units which control so many of the physical and physiological characteristics of animals. And, like the latter, it may be expected that they can be modified experimentally.

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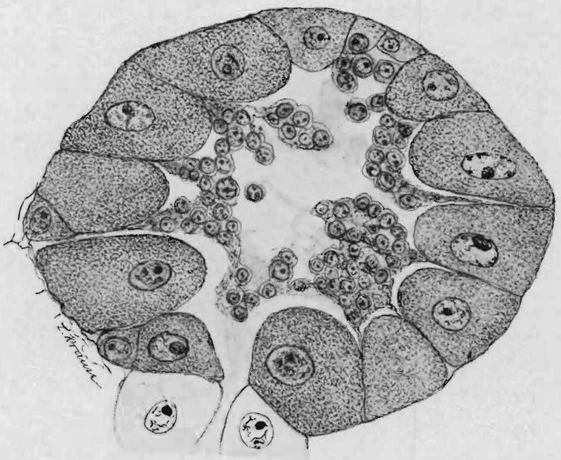
EXPLANATION OF PLATES

PLATE 7

Fig. 5. Transition from male to female phase; genital canal (*gc.*) filled with sperm-balls (*spz.*) ready to be discharged; a few spermatogonia (*spg.*) on the free surface of the large ovocytes anticipate the male phase which will follow ovulation; *ct.*, connective tissue in which gonad is imbedded.

Fig. 6. Climax of male phase in an older animal; the slender ovarian follicle is closely packed with cells in the later stages of spermatogenesis.

Fig. 7. Female phase immediately before ovulation; many spermatogonia in lumen of follicle. Numerous sperm-balls remained in the genital canals. February.



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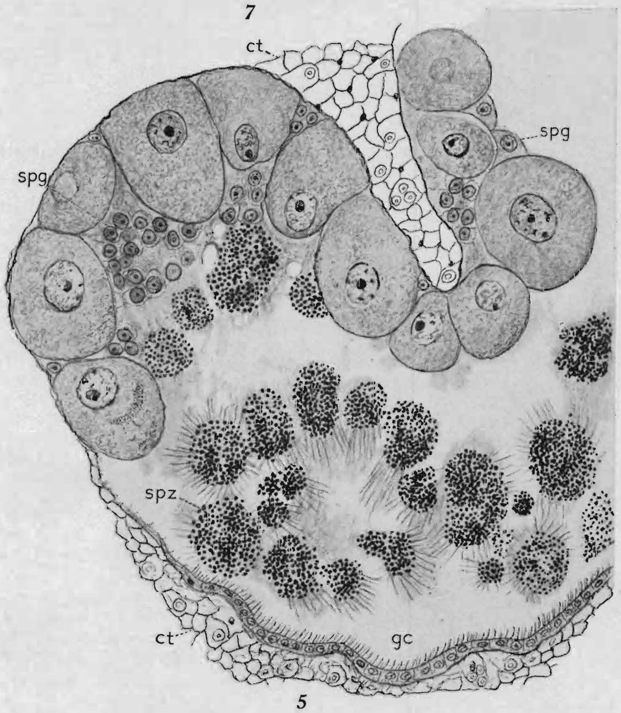

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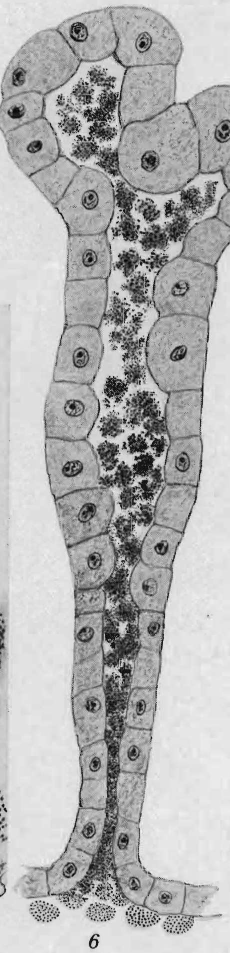

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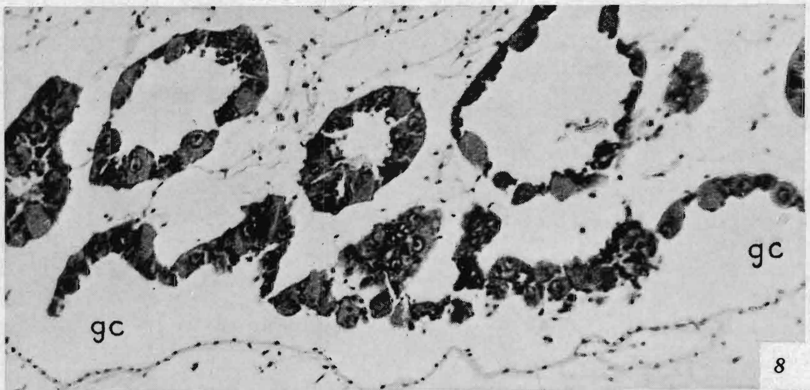
PLATE 8

Fig. 8. Portion of gonad at end of first female phase; from animal with segmenting eggs in mantle cavity. Note large, empty lumens with some ova still remaining on the walls. Spermatogonia have begun a rapid proliferation: *gc.*, genital canal. September 6–May 30, 38 weeks.

Fig. 9. Early second male phase with some ova (*ov.*) still loose in lumens and ducts, while spermatogonesis is proceeding. Larvae in mantle cavity. September 6–July 12, 44 weeks.

Fig. 10. Typical hermaphroditic phase; lumens and ducts crowded with cells in all stages of spermatogenesis, including ripe sperm-balls, as well as fully ripe ova. Both types of gametes are in such cases probably discharged simultaneously. February.



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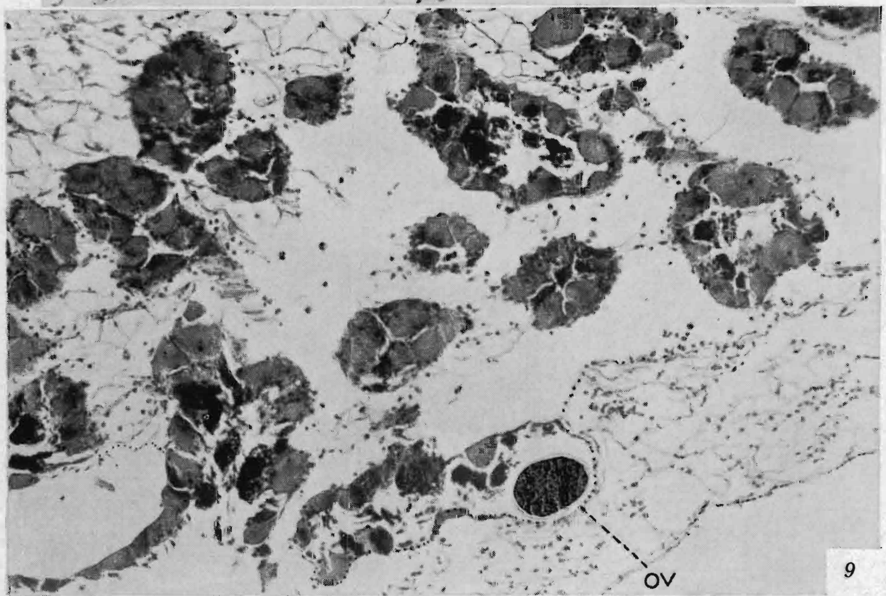

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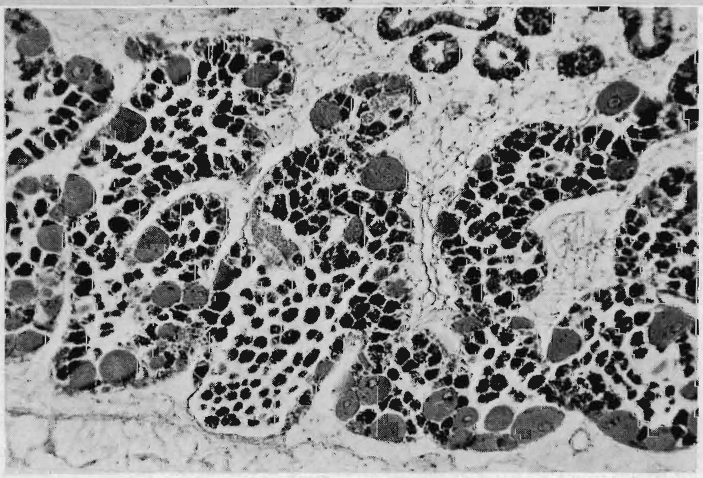

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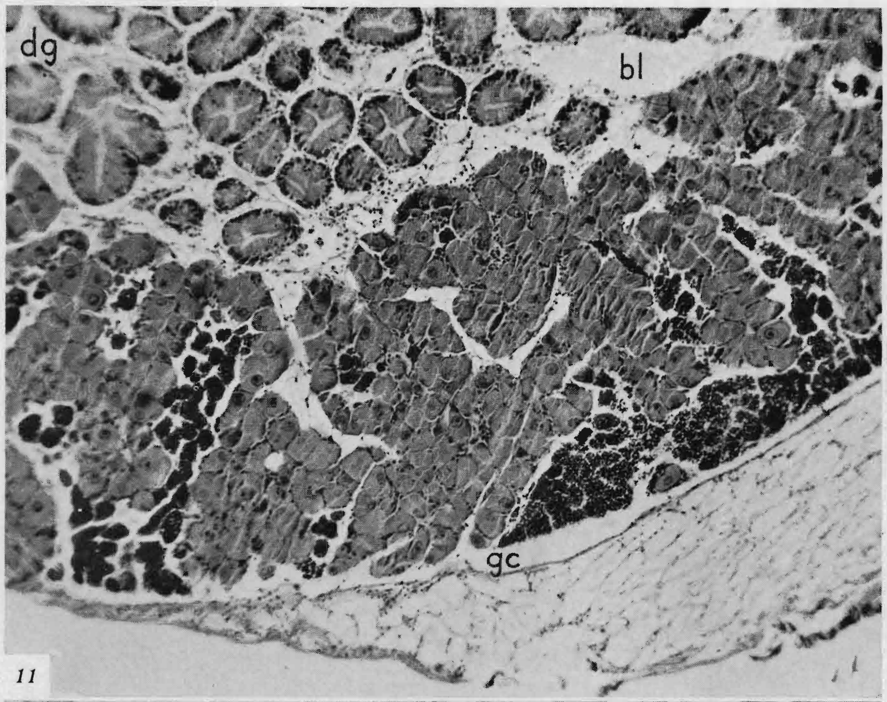
PLATE 9

Fig. 11. End of second male phase; lumens and ducts crowded with sperm-balls (dark in photo); ovarian tubules of secondary gonads with nearly ripe ova; *bl.*, blood vessel; *dg.*, digestive gland; *gc.*, genital canal. (September 6–June 14, 40 weeks.)

Fig. 12. Female phase of older individual with fully ripe ova. Small clusters of spermatogonia in lumens of ovarian tubules; letters as in fig. 11. February.



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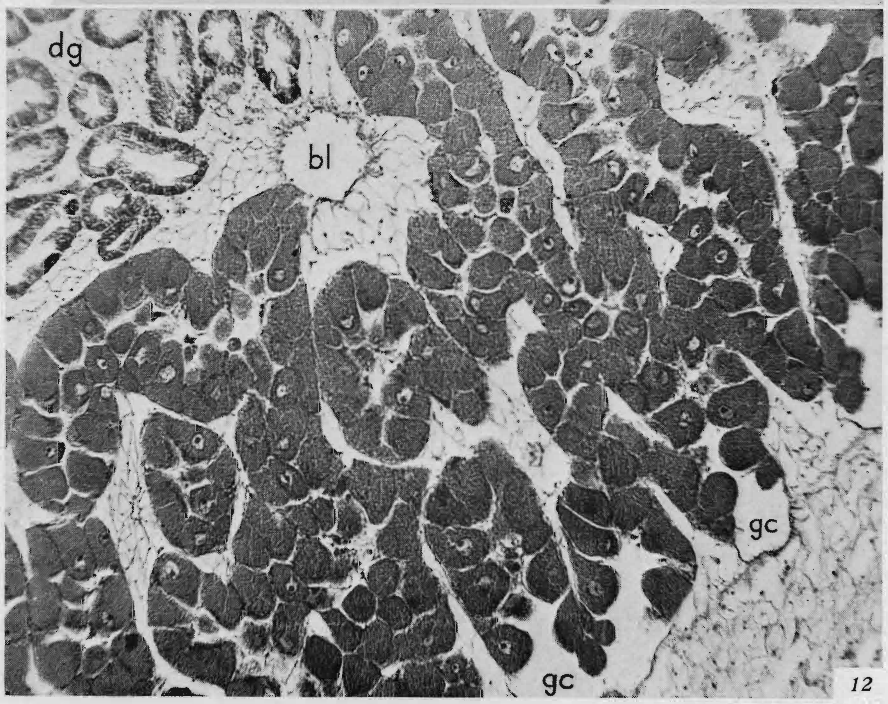

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