

BOARD OF NATURAL RESOURCES

State of Maryland

Department of Research and Education

OVARIAN GROWTH AND OVULATION IN
THE MATURE BLUE CRAB, *CALLINEC-*
TES SAPIDUS RATHBUN

W. L. HARD



No. 46

February, 1942

Chesapeake Biological Laboratory

Solomons Island, Maryland

State of Maryland
DEPARTMENT OF RESEARCH AND EDUCATION

Commissioners:

Lloyd M. Bertholf, *Chairman*.....Westminster
Julian D. Corrington.....Chestertown
E. N. Cory.....College Park
John T. Handy.....Crisfield
B. H. Willier.....Baltimore

Director:

R. V. Truitt.....College Park

Chesapeake Biological Laboratory:

G. F. Beaven, M.A., *Associate Biologist, Resident Manager*
Evelyn S. Beaven, M.A., *Assistant, Librarian*
Esther E. Dodd, *Secretary*
Ralph C. Hammer, B.S., *Assistant Biologist, Hatcheries*
R. A. Olson, Ph.D., *Associate Biologist, In Charge of Hydrography
and Pollution*
Harry Stern, B.S., *Assistant Chemist*
Willis L. Tressler, Ph.D., *Planktologist*
R. V. Truitt, Ph.D., *Biologist*

OVARIAN GROWTH AND OVULATION IN THE MATURE BLUE CRAB, *CALLINECTES* *SAPIDUS* RATHBUN*

W. L. Hard

Department of Zoology, University of Maryland

A. INTRODUCTION

Certain observations by Churchill (17-18) indicated that the female blue crab, *Callinectes sapidus*, may ovulate, or spawn, more than a single time during its mature life of from one to two years. How extensive this multiple ovulation occurs in the crab population as a whole has only been conjectured up to this time. The determination of the potential reproductive capacity of crabs promises to be of value for the purpose of estimating the brood reserve necessary to the reestablishment and maintenance of high levels of crab production.

A histological study of the ovary, at various periods during the life of the mature crab, revealed that two ovulations do occur. As a result of the histological study a method has been developed which utilizes the gross appearance of the ovary for determination of the stage in the reproductive cycle of the crab. The method has not only proved practical for determining the extent of ovulation in the crab population as a whole, but offers a means of securing other important information concerning the biology of this species.

The method of ovary examination, together with some reference to the nature of results obtained through its application, is herein discussed with the hope that it may be of value to biologists interested in the problems of crab conservation. A detailed report of the histological structure of the ovary during the reproductive cycle of the mature female crab will appear in a later publication.

B. OBSERVATIONS

I. The structure of the ovary during the periods of growth and ovulation in the mature crab.

1. *Condition of the ovary of a crab immediately following the last moult. Stage I.*

Careful examination of the medial wall of the spermathecae reveals very small anterior horns and transverse piece which compose the ovary

*This study was made possible in part by a grant from the College of Arts and Sciences of the University of Maryland.

at this stage (Fig. 1). The ovary is white in color in contrast to the typical orange color common to later stages. Some variation in the size of the ovary occurs in different crabs. Frequently the anterior horns are larger than those represented in Figure 1, but if so they are extremely delicate and can be identified only by careful dissection.

The female blue crab copulates only once, the act taking place when she moults for the last time and while in the soft stage at the start of her last instar (Truitt, 1939). Following copulation the spermathecae are tremendously distended with sperm suspended in a gelatinous fluid. The large, and somewhat pinkish, spermathecae are centrally located in the cephalothorax and are readily distinguishable from other visceral organs.

The ovary, at the time of the final moult, contains only small, immature eggs grouped in units which eventually make up the large follicles of the mature ovary (Fig. 8). A very active proliferation of ova occurs during this stage. These cells take their origin from a convoluted cord of germ cells which extends from the ovary proper to the periphery of the sperm sac (Fig. 8). The greater number of the new egg cells are in active mitotic division.

2. Stages in the maturation of the ovary. Stage II.

The greater percentage of immature crabs undergo their final moult and become mature in August and September. Churchill ('17-'18) observed that in some cases crabs which mature early in the summer may spawn that same year. However, this is undoubtedly of infrequent occurrence due to the late season in which they become mature and the further fact that during September spawning declines to an almost complete cessation. The usual cycle is for the crab to overwinter following copulation and to spawn the subsequent spring and summer. These observations are concerned with the more typical condition in which the crabs overwinter, following copulation, prior to the first ovulation. It is important, nevertheless, to realize that there is some variation in periods of time at which copulation, growth of the ovary, and ovulation occur.

The ovary increases greatly in size within a period of two months after copulation. This development is accompanied by a gradual decrease in size of the spermathecae. The typical stages seen during this period of time are reproduced in Figures 2, 3, and 4, in which the most conspicuous changes are in reduction in size of the spermathecae and the increased length in both the anterior and posterior horns of the ovary. The ovaries in these growth stages are orange in color, a

condition that is correlated with the maturation of the eggs and the formation of yolk. The eggs in early stages (Fig. 1) are without yolk granules (Fig. 8); thus the white color of the ovary in gross appearance.

The extent of ovarian growth occurring in the winter and early spring months can be visualized by comparing Figures 4 and 5. The conspicuous growth changes, from the condition of the ovary in late fall (Fig. 4) to its condition the following May (Fig. 5), are the increase in diameter and the more elongated posterior horns of the ovary, and a further reduction in the size of the spermathecae. The exact growth rate of the ovary has not been determined, but certain observations suggest that it proceeds in a gradual fashion over the winter and early spring months. The first ovulation, or spawning, usually occurs in May or June.

3. *Condition of the mature ovary immediately preceding the first ovulation. Stage III.*

Prior to ovulation, or the formation of the first sponge, the ovary develops to such a size and has such a pronounced orange color that it is the most conspicuous organ in the body (Fig. 7). It should be emphasized, however, that size alone, as a criterion, does not distinguish the unspawned crab from one in which a single ovulation has occurred, although it is possible to distinguish the ovaries before and after the first ovulation on the basis of their histological appearance (Fig. 9, 10).

The follicles in an ovary immediately preceding the first ovulation are considerably expanded owing to the development of large and mature eggs. The great amount of yolk present in mature eggs is, aside from size, the most striking characteristic of the ovary at this stage (Fig. 9). New egg formation occurs to some extent in the mature ovary, as seen by an occasional cord of immature follicles (Fig. 9).

4. *Condition of the ovary between the first and second ovulation. Stage IV.*

The ovary in a crab which has spawned once may be, as indicated in the preceding section, fully as large as the ovary was before ovulation took place (Fig. 5). Usually some decrease in size occurs but, since there is considerable variation in size of the mature ovary, it is not possible to distinguish the ovulated ovary on the basis of size alone. Some cases were noted in which the ovary was reduced in diameter, following the first ovulation, until it approximated a size that occurred in the growth period (Fig. 4).

The postovulated ovary may be distinguished from the ovary of the

unspawned crab, whether mature or immature, by histological examinations. Immediately after the first ovulation the ovary is still filled with eggs most of which are fully as large as those in a mature ovary but they lack the great quantity of yolk characteristic of the mature egg. The relatively small amount of yolk present is sufficient to give an orange color to the ovary. The areas from which eggs have been discharged represent, however, the most conspicuous characteristic of the ovulated ovary (Fig. 10). These areas usually appear in the center of the ovary, although occasionally at its periphery, and are entirely, or partially, filled with some globular material which is prominently stained with iron hematoxylin. The origin and nature of this substance is not known but its presence in the ovary following the first ovulation, but not the second, renders it of considerable interest and of diagnostic value in determining the occurrence of ovulation.

It is impractical to resort to histological methods to distinguish the stage of reproductive activity of the ovary in routine examinations suitable for field work. Fortunately other criteria may be used, but heretofore they have not found application since the histological observations have not been available to correlate with the gross appearance of the ovary in order to determine the stage of the reproductive cycle. Churchill ('17-'18) clearly demonstrated the method of examining the swimmerets for egg cases as a means of determining whether or not the crab had previously spawned. The presence of egg cases make it possible to determine that a crab has spawned but, as will be discussed later, this evidence is not sufficient to determine whether or not the sponge was the first or the second to be released. Ovary examination together with identification of sponge remnants is necessary to determine the number of ovulations which have occurred.

In application, then, the presence of a full ovary (Fig. 5) in a crab which has no egg cases on the swimmerets should be taken to indicate a crab not, as yet, having spawned; that is, either a growth stage in the maturation of the ovary (Fig. 4) or, if of larger size, a mature ovary ready for ovulation (Figs. 5, 7). On the other hand, the presence of a full ovary with egg cases on the swimmerets designates the crab as one which has ovulated once. These observations were confirmed by histological studies of the ovaries before and after ovulation (Figs. 9, 10).

5. Condition of the ovary after the second ovulation. Stage V.

Following the second ovulation the ovaries are small compared to

the mature ovary, and usually of a grey or brownish color (Fig. 6). A few cases were observed in which isolated areas of the ovary exhibited the orange color typical of the full ovary thereby indicating areas from which mature eggs had not been discharged. The number of mature eggs present in such ovaries is entirely insufficient to provide for a third sponge.

The ovary in Stage V characteristically exhibits a very loose arrangement of relatively few eggs which, for the most part, are immature as indicated by their small size and the absence of yolk (Fig. 11). The follicles are reduced in size, as may be seen by comparing Figures 9 and 11. A great amount of connective tissue serves to separate adjacent eggs in contrast to their compact arrangement in the mature ovary (Fig. 9). Some proliferation of new egg cells occurs after the second ovulation. This might indicate a regeneration of the ovary to a functional capacity, but there is substantial evidence to indicate that the eggs are destined to degenerate. Frequently specimens are observed in which all the newly formed eggs within a follicle are in advanced stages of degeneration. The larger eggs, of the size prominent in Figure 11, likewise show all degrees of degeneration in a single ovary. Typically, the ovary, after the second ovulation is entirely depleted of mature eggs, and it is extremely doubtful, considering the extensive, and probably total degeneration of ova, that the ovary ever recovers to a condition in which it is capable of reproductive activity.

The collapsed ovary (Fig. 6) approximates, on a basis of size, certain of the growth stages (Figs. 3, 4). Distinctions between the stages easily may be made on gross appearances. The exhausted ovary is usually grey or brownish in color while the maturing ovary is invariably orange. A final and infallible distinction may be made by examining the swimmerets for remnants of a sponge, which, of course, would be absent from crabs in which the ovaries are immature. A comparison of Figures 5 and 6 shows the significant decrease in size of the ovary following the second ovulation as compared with its condition before or after the first ovulation.

There is no recognizable difference in the size of the spermathecae during and following the periods of spawning. It is evident from histological studies of spermathecae that sufficient spermatozoa are placed in the spermathecae during the one copulation period to assure fertilization of all eggs released during the life of the crab. Considering the time interval between copulation and ovulation it is evident that the sperm remain viable for a period of about eighteen months.

Further, it has been established (Sette and Fiedler '25) that the spermathecae of the mature crab, including the senile individuals, virtually always contain sperm. It has been reported (Truitt)* that the mature female crab will initiate but not complete another shedding if copulation is not effected at the time of the final or mature moult. Among individuals observed in the hard crab catch and the vast thousands maintained on shedding floats in the industry, there is record of only three mature female crabs having been observed in the peeler condition; and hence unmated specimens (Truitt).* Since the spermathecae of the mature female crab always contain sperm, and in sufficient number to assure fertilization of all eggs produced, it is evident that the reproductive capacity of the crab is dependent solely on the activity of the ovary in producing eggs. Thus, to determine the extent of spawning, it is necessary to examine the condition of the ovary, in terms of egg production, rather than to estimate spawning from the appearance of the spermathecae as was attempted by Sette and Fiedler ('25).

C. DISCUSSION AND CONCLUSIONS

The histological structure of the ovary in the mature crab has been correlated with (1) the size and appearance of the ovary and (2) the presence or absence of egg remnants on the swimmerets. These observations have resulted in the development of a method which is suitable for routine analysis of the reproductive capacity of a crab population and one that is applicable to certain other problems of crab biology.

- Stage I. Immediately following the last moult. Ovary small, inconspicuous, white in color; spermathecae greatly distended due to recent copulation (Fig. 1).
- Stage II. Growth period of the ovary, from the time of copulation to the time of ovulation, typically from August to May. Ovaries orange in color and marked by a gradual increase in length and diameter with an accompanying decrease in size of the spermathecae (Figs. 2, 3, 4).
- Stage III. Mature ovary preceding the first ovulation. Ovary bright orange in color and of large size (Fig. 5). Distinguished from Stage IV by the absence of sponge or egg shell remnants on swimmerets.
- Stage IV. Period between first and second ovulations. Ovaries orange in color and of large size, although sometimes

*R. V. Truitt, Personal communication, August, 1941.

smaller than in the preceding stage, but size difference being insufficient to distinguish Stages III and IV. Sponge or egg shell remnants present on the swimmerets (Figs. 5, 7).

Stage V. Following the second ovulation. Ovary collapsed, grey or brownish in color (Fig. 6). Sponge or egg shell remnants present on the swimmerets.

Observations have been made on a sufficient number of samples (Table I) to demonstrate the accuracy and facility with which the above characteristics, constituting a method, may be used to determine the degree of maturity of crab ovaries and the relation of same to spawning. Likewise, observations were made to determine the nature of technical difficulties which might be experienced in the application of this method. In examining crabs for remnants of egg shells, as an indication of a previous sponge, it is not accurate to base a conclusion solely on the discoloration of the swimmerets or, for that matter, on the appearance of the crab as a whole. Crabs have been observed which, from the discoloration of the swimmerets, as well as the "weather beaten" appearance of the exoskeleton, would indicate an exhausted condition, or at least individuals which had spawned once; yet ovary examinations revealed such crabs to be in Stage II and, hence, not spawned at all. It is necessary to examine the swimmerets under low magnifications (10X to 30X diameters) and specifically identify either the remnants of the egg shell or the stalks by which the eggs were attached to the swimmerets. Many times only a very few remnants of the sponge are to be found, but with practice and careful observation it is possible to become adept at identifying these characteristics. Best results are obtained through selection of the most anterior swimmerets which should be spread out in water so individual filaments may be seen distinctly.

The most significant direct result obtained from this study is the demonstration that a very high percentage of mature crabs spawn twice. This information is of significance in determining the reproductive capacity of the crab population as well as in offering evidence of value in establishing a necessary brood reserve. Previously, Churchill ('17-'18) and Sette and Fiedler ('25) had confined crabs on floats, or in pens, and observed that in a few cases two ovulations occurred. Just how extensive multiple ovulations occurred in the crab population as a whole could not be determined from the few observations made, or by the method used.

TABLE I
The figures are quoted in percentages of
the total female crab population.

| Date | Locality | Stage of ovary development | | | | | % of crabs sponged |
|-----------|-----------------|----------------------------|------|-----|------|------|--------------------|
| | | I | II | III | IV | V | |
| July 19 | Lynnhaven Inlet | 0 | 5 | 13 | 45 | 37 | 82 |
| July 30 | " | 0 | 2 | 0 | 58 | 40 | 98 |
| August 12 | " | 0 | 4.6 | 1.7 | 38.4 | 55.3 | 93.7 |
| August 22 | Little Bay | 1 | 0 | 1 | 10 | 88 | 98 |
| October 8 | Mobjack Bay | 79.1 | 19.4 | 0 | 1.5 | 1 | 2.5 |

The highest percentage of crabs that had ovulated twice was taken from Little Bay on August 22 (Table I). Eighty-eight per cent of the female crabs were either carrying the second sponge or remnants of it. Of the remainder, ten per cent of the crabs were in Stage IV. Considering the variations in time of ovulation there is reason to believe that this ten per cent would continue with another ovulation and thus make a total of ninety-eight per cent of the female population ovulating twice. This second ovulation, on the part of the ten per cent indicated above, may occur in the fall or the following spring. Mr. Beaven, of the Chesapeake Biological Laboratory, has kindly offered his observation that the peak of the first spawning, that is in May or June, is usually higher than the peak of the second period of spawning in August. The increased number of sponge crabs in the spring possibly may represent crabs which spawned only once the preceding year, while the remaining number are mature females spawning for the first time. This point can be determined by continuing the ovary examinations throughout a yearly cycle. Records of the percentages of male crabs, although available, have not been included in this report since they are of no significance to this subject for females, virtually without exception, were observed to have been mated.

The complete degeneration of the eggs remaining in the ovary following the second ovulation (Fig. 11) indicates that the crab has finished its reproductive activity.

Some interesting lines of investigation are suggested by the results of the observations made thus far (Table I).

First, ovary examinations made at monthly intervals over a twelve-month period would be of great value in (1) showing the predominant type of crab at any given time in the area selected and (2) in showing both the real and potential reproductive capacity of the crab population over a given period of time. A shift from stages III to V is noted in crabs collected at Lynnhaven from July 19 to August 12 (Table I). Thus, as the season progresses an increased number of the crabs in this

area become exhausted and the reproductive capacity becomes progressively lower.

Secondly, by establishing a number of sampling stations at convenient locations along the Bay it would be possible to get an accurate picture of both the courses of migration and the type of crab in a given area within limited periods of time, as indicated by the high percentage of newly matured crabs, stages I and II, in Mobjack Bay in October (Table I). Although exact figures for this area are not available it is certain that during the summer months a high percentage of sponge crabs, in stages either IV or V, or in both, is found. A migration of crabs, in stages IV and V, has undoubtedly occurred out of this area and the population is replaced by a new migration of crabs which show, in practically all cases, immature stages of ovary development.

A third, and perhaps the most significant, application of this method of sampling crabs is that it offers an accurate means for the determination of a brood reserve. It may be handled in an identical manner as are scale studies in determining a spawning reserve in a fish population. A correlation of fishing intensity, as obtained from catch records, with the relative percentages of crabs in various stages of the reproductive cycle offer an index to the extent of brood reserve necessary to maintain a given crab population. The method might also serve to designate the areas which would be most suitable for the maintenance of this brood reserve. Heretofore there has been no method applicable to the problem of determining the brood reserve necessary to maintain a high level in crab production.

D. ACKNOWLEDGMENTS

This study was initiated, and the major part of the work completed, at the Chesapeake Biological Laboratory during the summer of 1941. Opportunity is taken at this point to express indebtedness and appreciation to Drs. L. B. Broughton and R. V. Truitt for providing funds, materials, and field and laboratory facilities necessary for the pursuance of this work. The latter originally pointed out the possibilities of the study and offered every encouragement to the pursuit of the problem. Also, the author sincerely appreciates the aid and advice rendered by Messrs. R. A. Nesbit and G. F. Beaven on numerous phases of the work. The assistance of L. E. Cronin and K. E. Goellner, who collected the specimens and made certain ovarian examinations, is gratefully acknowledged.

E. BIBLIOGRAPHY

- CHURCHILL, E. P., JR. 1917-18. Life history of the blue crab. *Bulletin of Bureau of Fisheries*; Vol. 36, pp. 93-134.
- SETTE, O. E. and R. H. FIEDLER. 1925. A survey of the condition of the crab fisheries of Chesapeake Bay. *Bureau of Fisheries Report*.
- TRUITT, R. V. 1939. Our water resources and their conservation. *Chesapeake Biological Laboratory, Cont. No. 27*.

PLATE I
Explanation of Plate

Figures 1 to 6 are somewhat reduced from normal size but they were photographed at the same level, hence the same degree of reduction is represented throughout these figures.

Figure 1. Ovary (ov) and spermathecae (sp) of a crab shortly after its final moult. The spermathecae are distended with sperm as a result of copulation which occurred at the time of the final moult.

Figures 2, 3 and 4. Growth stages in maturation of the ovary. The stages, including Figure 1, represent approximately a two month interval following the final moult. Note the gradual increase in length and diameter of the ovary and the decrease in size of the spermathecae.

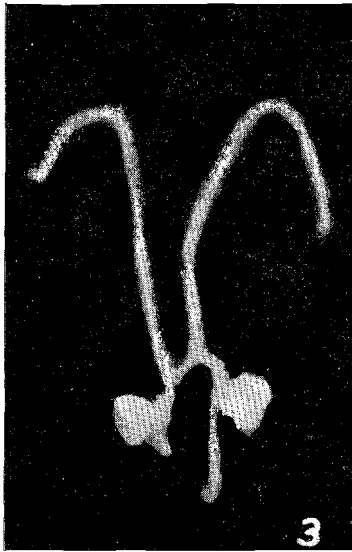
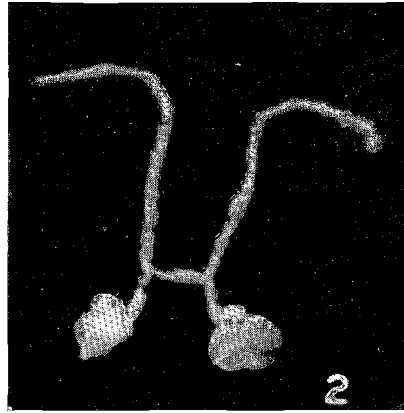
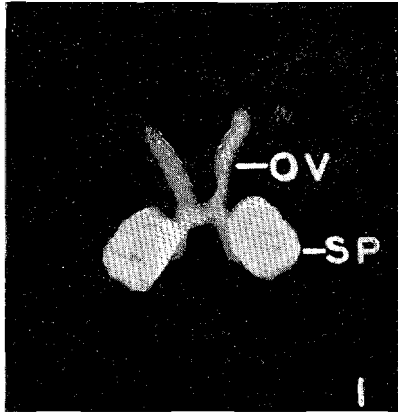


PLATE 1

PLATE II
Explanation of Plate

- Figure 5. Ovary after the first ovulation. Note the increased diameter and length of the posterior horns of the ovary as compared with that of Figure 4.
- Figure 6. Ovary following the second ovulation. The decrease in size as compared with the ovary following the first ovulation (Fig. 5) is apparent.
- Figure 7. Carapace removed from the crab to show the location of the ovary (ov). The spermathecae are ventral in position and hidden from view. This crab had spawned once. The ovary is of a size comparable to that of Figure 5.

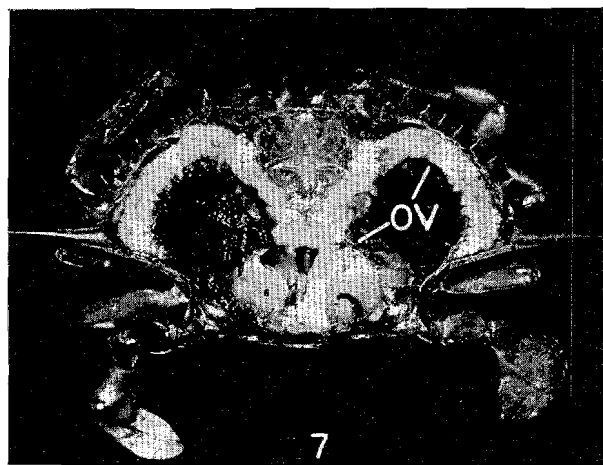
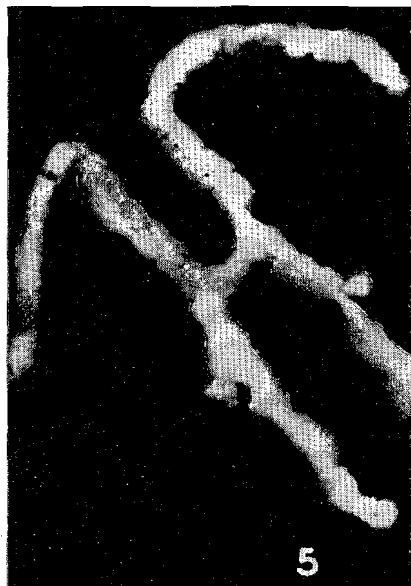


PLATE 2

PLATE III
Explanation of Plate

- Figure 8. Section of the ovary of a peeler crab, similar in size to the ovary shown in Figure 1. Compare the egg size with those in the mature ovary (Fig. 9). sp., the very outer edge of the spermatheca; pc., germ cell cord from which new egg cells are produced. X 110.
- Figure 9. Portion of one follicle in a mature ovary preceding the first ovulation. The large eggs are filled with yolk granules. A few immature eggs are present in this section. X 110.
- Figure 10. Section through the center of an ovary following the first ovulation and showing the large areas from which eggs have been extruded. X 110.
- Figure 11. Section of an ovary following the second ovulation. Note the small follicles containing a few immature eggs which are widely separated by connective tissue. Compare with the mature ovary in Figure 9. X 110.

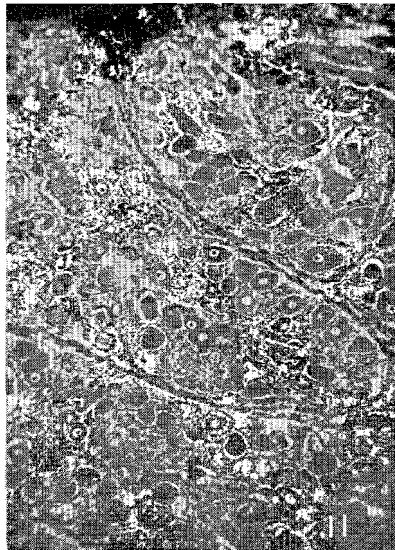
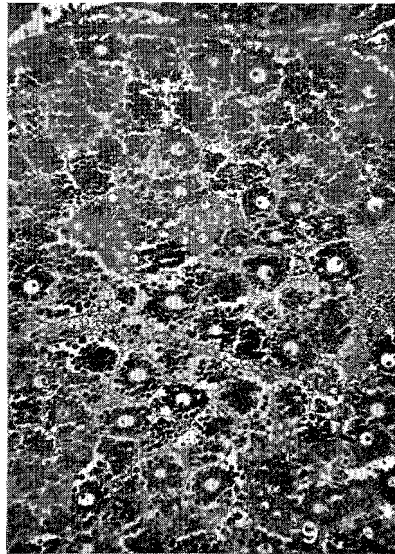
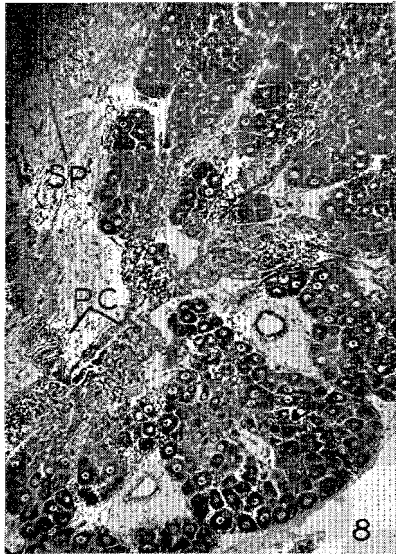


PLATE 3