Northwest Atlantic Fisheries Organization

Serial No. N136

NAFO SCR Doc. 80/VI/82

Comparative histological and visual observations on oogenesis and sexual maturity of the Greenland Halibut in Northern Labrador waters

by

S. J. Walsh and W. R. Bowering
Department of Fiheries and Oceans
P.O. Box 5667
St. John's, Newfoundland
A1C 5X1

ABSTRACT

Histological stages of ovaries of female Greenland halibut are compared for accuracy with maturity stages of observers aboard research vessels. There was little difference between estimation of 50% length-of-maturity ogives for both histological and visual observations and ovaries. A relationship between percentage degeneration of young oocytes in immature fish and 50% length-at-maturity ogives is seen.

INTRODUCTION

The Greenland halibut, (Reinhardtius hippoglossoides (W)) spawns during winter in the deep warm waters between Canada and Greenland (Templeman, 1973). Templeman concluded that higher temperatures aid ovarian and embyronic growth and that halibut with large eggs close to spawning condition are extremely uncommon in Newfoundland's east coast bays, where bottom temperatures of the deep water are lower than in similar depths on the continental slope.

Greenland halibut has become one of the most important fisheries on Canada's east coast in recent years. Assessments of this stock (Bowering, MS 1979) have indicated that this fishery is prosecuted almost entirely on the immature section of the stock. With the lack of mature fish in the catches until very large and quite old, it was considered that the visual observations on the maturity of the females may be misinterpreted in that resorption of spent fish may be very rapid. Consequently, the ovaries may appear immature when in fact they may have spawned previously. With this is mind, a comparative histological and visual study was initiated in order to elucidate the problem.

Also, the estimation of percentage ratios of ovarian maturity in female fish aids in the understanding of the sexual cycle and the onset of spawning in the Greenland halibut. The accuracy of fishery scientific observers in estimation of ovarian maturity is very critical to this understanding.

This paper compares the descriptive stages used for visual observations with histological analysis of ovaries. Maturity ogives for visual and histological observations are given. Preliminary observations on oogenesis are reported for the Greenland halibut in ICNAF Subdivisions 2G and 2H.

MATERIAL AND METHODS

Female Greenland halibut gonads were collected in August 1979 from ICNAF Div. 2G, 2H on a stratified random survey cruise of the Research vessel Gadus Atlanitica, operated by Northwest Atlantic Fisheries Centre. Samples of fish were taken in a depth range of 370-752 m with a bottom temperature range of 0.7°-4.1°C.

One hundred thirteen whole ovaries were collected from fish 50 cm and up, allowing a maximum of 11 gonads per 5 cm group. Ovaries were assessed visually for stages of maturity and then placed in 10% buffered formalin* (p.H.7.0). Pieces excised from posterior portion of the the ovaries were dehydrated through alcohols and embedded in paraffin wax. Sections were cut at 6-8 microns and stained with Harris' Haematoxylin and Eosin and a variant of Mallory's Triple. Microphotography was done on a "Zeiss Photomicroscope III" at 78.75X magnification.

Descriptive visual maturity stages for each ovary are compared to histological stages used by Fedorov (1968) for Greenland halibut in the Barents Sea. Maturity ogives for both types of observations are given using 4 cm length groupings.

RESULTS

The length distribution of the sample ranged from 50 cm to 113 cm. Each ovary was analyzed for development under the microscope using Fedorov's (1968) maturity stages for the Barents Sea Greenland halibut (Table 1). Most of the ovaries were in stage 2** (53.98%) immature and stage VI-III (34.51%) spent and re-maturing (Table 2) (Plates I, II). Due to the time of the year (August) stage IV, the yolk filled stage, and stage V, the 'running stage' were absent. The juvenile or stage I was also absent due to no fish under 50 cm being sampled.

Evidence of mass degeneration and reabsoprtion of older oocytes entering early stage III, the beginning period of vacuolation and primary yolk accumulation was evident in many immature fish (Plates III-VI). Fedorov (1968, 1971) detailed this occurance in Greenland halibut of the Barent's Sea and concluded that oocyte resoprtion occurs with the active participation mainly of follicular cells. Emigration of freely wandering elements, possibly phagocytes, into the oocyte is observed during the beginning period of degeneration (Plate III). These degenerating oocytes stained heavily with haematoxylin stain while normal oocytes are stained by eosin.

* As a result of several days storage in formalin and subsequent storage in 70% alcohol distortion of the shape of older oocytes was evident.

** When the word "stage" is used, it refers to Fedorov's histological stages of maturity.

This resportion of older oocytes is evident in immature fish found from 54 cm to 93 cm. A plot of the percentage of maturity from histological analysis against the percentage of fish undergoing resorption in early stage III (Table 5, Fig. 1) indicates that when fish reach the 75-80 range only 50% of the immature fish exhibit oocyte degeneration. Conversely, it is at this size range that fish are reaching 50% maturity.

No evidence of sterility or degeneration of oocytes in sexually mature females was noted. Further sampling is needed to examine whether degeneration of sexually mature oocytes occurs as Fedorov (1971) reports for Barents Sea Greenland halibut.

Accuracy of Visual Estimates of Maturity

The accuracy of observers in appraisal of the stages of maturity for female ovaries was tested by comparing visual observations with that of histological analysis (Tables 1,3). Inaccuracies were found in visual estimation of maturity stages, but were not numerous enough to affect the overall estimation of maturity ogives (Table 4). This is evident from a plot of percent maturity from visual observation against the percent maturity from histological analysis (Table 5 Fig. 2). For the overall size groups M-50% falls in the size range of 75-80 cm for both analyses. However, visual estimation of different stages of maturity should not be considered solely accurate in defining the sexual cycle of Greenland halibut. As seen in Table 4 occurance of degeneration of oocytes in early stage III led the observers to confuse these immature fish as: mature fish, spent fish and spent fish that are maturing again. Since ovaries of fish that have completed spawning may transfer rapidly to stage III, 70% of the samples were mistaken for fish which are reaching spawning condition for the first time.

DISCUSSION

Templeman (1973) notes that the presence of Greenland halibut in ICNAF Div. 2G, 2H is often related to spawning on the continental slopes and to migrations to and from the spawning grounds. Upon maturity the Greenland halibut migrates to the deep edge of the continential slope zone whereas immature fish are found on the shelf and deep bays (Zilanov et al. MS 1976).

From the ovaries collected on the shelf of Div. 2G and 2H preliminary histological analysis along with visual analysis shown that the sample was made up of immature fish and fish that had spawned the previous winter. The occurrence of large scale reabsorption of oocytes just beginning vacuolation and primary yolk accumulation (Stage III) can inhibit the subsequent course of oogenesis and disrupt the reproductive rhythm (Fedorov 1968, 1971). This may be a suitable explanation for the Greenland halibut not starting maturity until a size range of 75-80 cm is reached. As the percentage of ovarian maturity increases the percentage of degeneration of early stage III decreases.

The accuracy of the observer interpretations on different stages of ovarian maturity can be enhanced by histological analysis. The passage of immature fish into maturing for the first time; and confusion of fish that have spawned and are maturing again with fish maturing for the first time are the sources for error in this sample. However, maturity ogives for both visual and histological observations, for practical purposes, are in agreement with each other.

CONCLUSIONS

1. Ovaries exhibiting mass scale resbsorption of oocytes entering Stage III appears to disrupt ovarian maturation and prolongs the immature stage.

2. The accuracy of observers can be greatly enhanced by use of histological analysis.

3. Maturity ogives for visual and histological observation of ovaries are very much in agreement.

4. Further sampling is needed to give a more accurate picture of oogenesis and the sexual cycle.

REFERENCES

Bowering, W. R. MS 1979. The Greenland halibut fishery on the Continential Shelf of ICNAF Subarea 2 and Divisions 3KL. ICNAF Res. Doc., No. 79/VI/71, Ser. No. 5413.

Fedorov, K. Ye. 1968. Ovogenez i polovoi tsikl chernogo paltusa Tr. Polyarn. Nauchno-Issled. Proektn. Inst. Morsk. Rybn. Khoz. Okeanogr. 23: 425-450.

1971. The state of the gonads of the Barents Sea Greenland halibut [Reinharditus hippoglossoides (Walb.)] in connection with failure to spawn. J. of Icthyology, Vol. 11, No. 5.

Templeman, W. 1973. Distribution and abundance of the Greenalnd halibut, Reindarditus hippoglossoides (Walbaum) in the Northwest Atlantic.

Zilanov, V. K., A. A. Strogano, F. M. Troyanovsky and A. K. Chumakov. MS 1976. The results of the study of the commercial reserve of Greenland halibut at the continental slope in the Northwestern Atlantic. ICNAF Res. Doc., No. 109, Ser. No. 3932.

Table 1. Descriptive stages of maturity and Fedorov's (1968) histological stages for ovaries of Greenland halibut.

	Visual stages		Histological (Fedorov's Stages
1A	Juvenile stage: ovary is very small no signs of egg development.	1.	Oocytes in protoplasmic growth stage, nests of oogonia and oocytes present. Ovarian wall thin (Juvenile stage).
1B	Immature: ovary small, pinkish. Eggs not visible to naked eye. Ovarian wall thin and transparent.	2.	Monolayer follicle phase in primary oocyte development with stage 1 present.
2.	Maturing A: Eggs becoming visible to the naked eye. All eggs opaque. Ovarian wall thin.	3.	A. Early stage 3, oocyte entering trophoplasmic growth, with beginning vacoulation and primary yolk accumlation. Chorion (zona radiata) visible.
3.	Maturing B: Mixture of opaque and clear eggs with less than half bulk of eggs clear.	3.	B Late stage 3 oocytes enter intensive trophoplasmic growth and heavy yolk deposition, Mixture of early and late stage 3 and some stage 1 and 2.
4.	Maturing C: Mixture of opaque and clear eggs with more than half of the bulk of eggs clear. This category includes the ripe condition where contents are almost liquid with translucent eggs.	4.	Most developed oocytes are filled with yolk or are completing growth. The following generation of oocytes are in vacuolation and primary yolk accumulation. Radial strations appear in the chorion (zona radiata).
5.	Running stage: (Partly spent) some eggs extruded but several thousand clear eggs remaining.	5.	Eggs have hydrated and the appearance of flowing sexual products is noted . Commencement of spawning is ready to begin. (ovulation) Older generation oocytes are entering periods of maturation.
6.	Spent Stage: Ovary appears redish purple in appearnace. Ovary well is thick and tough. Some residual clear on opaque eggs are seen. This stage passes gradually into maturing a condition.	6.	Ovary contains oocyes of new generation in the phase of vacuolation and primary yolk accumulation (Stage 3) and the entire complex of sexual cells of Stage 2 are present. Ovarian wall is thick. Large number of ruptures follicles and unreleased oocytes are undergoing resportion. (Stage 6-3). The latter part of this stage is sometimes referred as the "resting stage".

Table 2. Comparison of visual and histological analysis of the stages of maturity for 113 ovaries.

Immature Stage II		Maturing Stage III-IV		Spent Stage VI		Spent and Maturing Stage VI-III	
Visual	Histology	Visual	Histology	Visual	Histology	Visual	Histology
55	61	11	5	8	8	38	39
48.67%	53.98%	9.73%	4.42%	7.08%	7.08%	33.63%	34.51%

Table 3. Comparison of accuracy of observers with histological analysis of ovaries of 4 cm groups. N.B. Due to small numbers in the sample 98-113 cm groups are combined.

Length (cm) No. in parenthesis	Immature		Maturing		Spent		Spent-Maturing	
	Visual	Histology	Visual	Histology	Visual	Histology		
50-53 (5)	5	5	0	0	0	0	0	0
54-57 (9)	9	9	0	0	0	0	0	0
58-61 (11)	10	9	0	1	0	0	0	0
62-65 (9)	8	8	1	1	0	0	0	0
66-69 (8)	7	8	1	0	0	0	0	0
70-73 (10)	8	9	0	1	0	0	1	0
74-77 (10)	4	7	0	0	3	0	3	3
78-81 (9)	1	3	2	1	2	1	4	4
82-85 (10)	1	1	2	1	2	1	5	7
86-89 (10)	0	1	2	0	1	1	7	8
90-93 (9)	0	0	1	0	0	2	7	6
94-97 (7)	0	0	1	0	0	1	6	6
98-113 (8)	0	0	1	0	1	1	6	6

Table 4. List of errors in observers estimations of maturity and their correction by histological analysis.

Stage	Actual No. by visual analysis	Corrections by Histological analysis
Immature	55 ovaries	54 observations correct; 1 fish was maturing (Stage 3)
Maturing (first time) condition	11 ovaries	1 observation correct; 2 ovaries were immature, 1 ovary mature, 7 ovaries had spawned previously and were maturing agan.
Spent condition	8 ovaries	2 observations correct; 3 ovaries were immature, 1 ovary was maturing, 2 ovaries were spent and maturing again.
Spent-maturing	38 ovares	29 observations correct; 1 ovary was immature, 2 ovaries were maturing for first time and 5 ovaries were in the spent condition and "resting".

Table 5. Percentage of length-at-maturity stages from visual and histological analysis and percentage of length groups showing deneration of oocytes entering stage III.

Length (cm) actual nos. in ( )	% Mature visual	% Mature histology	% Showing degeneration of oocytes
50-53 (5)	0	0	0
54-57 (9)	0	0	55.6%
58-61 (11)	0	9%	81.8%
62-65 (9)	11.1%	11.1%	66.67%
66-69 (8)	12.5%	0	100%
70-73 (10)	20%	10%	90%
74-77 (10)	60%	30%	70%
78-81 (9)	88.89 %	66.7%	44.4%
82-85 (10)	90%	90%	10%
86-89 (10)	100%	90%	10%
90-93 (9)	88.89%	88.9%	11.1%
94-97 (7)	100%	100%	0
98-113 (8)	100%	100%	0

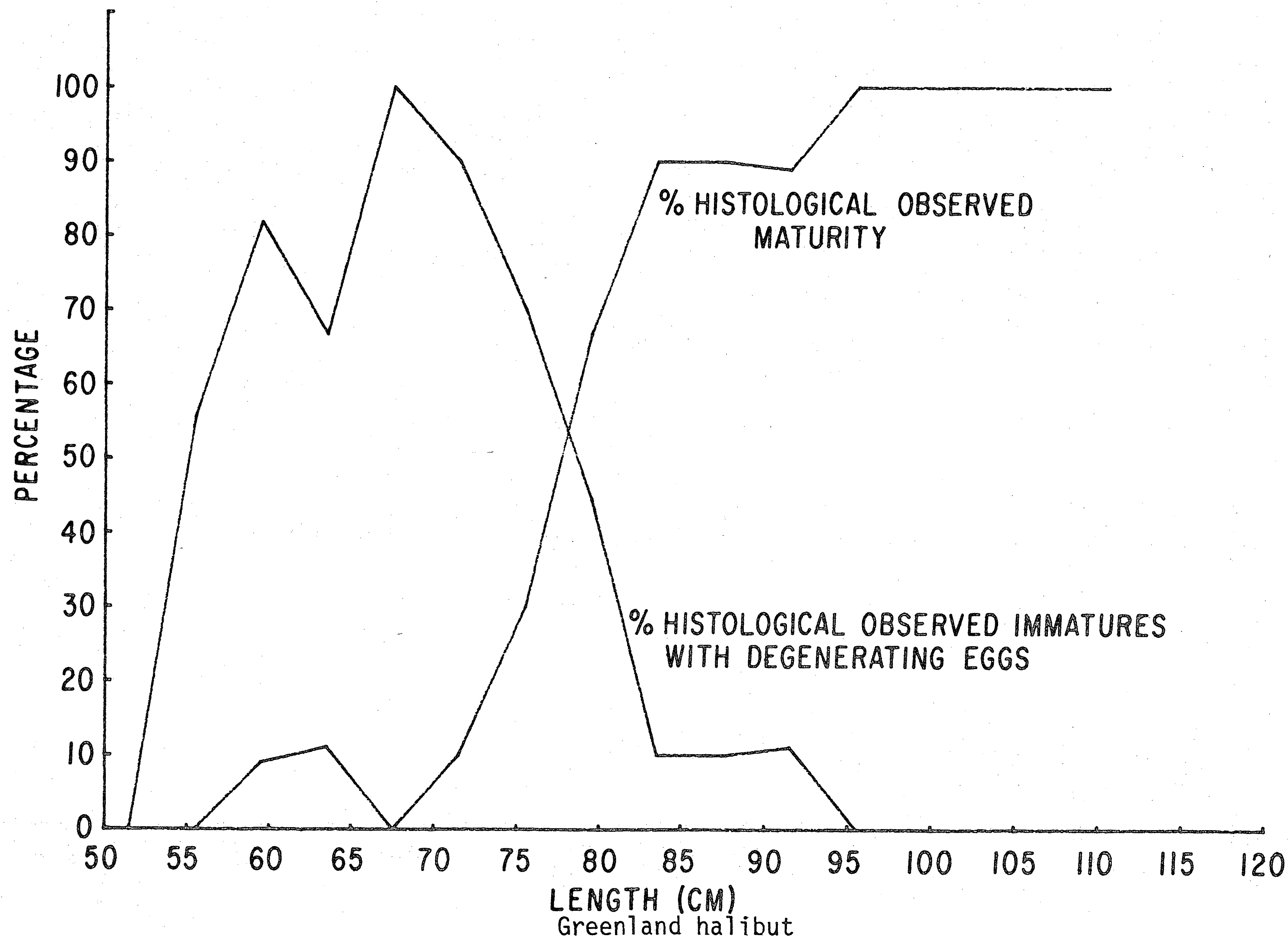


Fig. 1. Plot of % of immature fish exhibiting mass scale degeneration of oocytes entering early stage III and a maturity ogive from histological analysis.

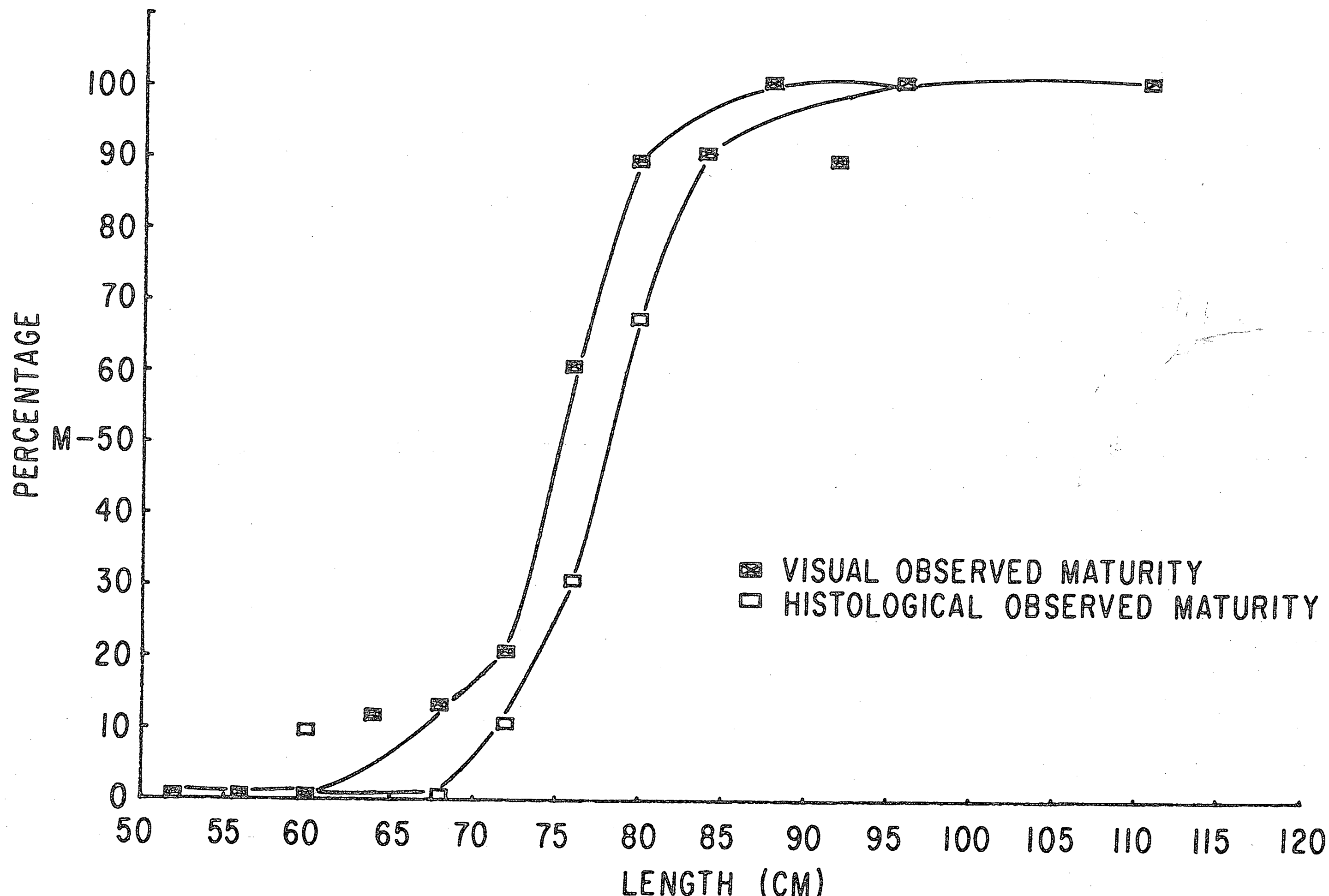


Fig. 2. Maturity ogives for visual and histological stages of maturity.

Explanation of Plates

Histology of Greenland halibut ovaries (Haematoxylin and Eosin)
objective 6.5X, ocular 12.5X

Plate I: Stage I-II; Immature stage, showing oogonia and oocytes in monolayer follicular growth. Length of fish is 51 cm.

Plate II: Stage VI-III; Spent and maturing again (a) oocyte beginning vacuolation and primary yolk accumulation; (b) oocyte in intensive yolk accumulation; (c) remains of reabsorped follicles from previous spawning. Length of fish is 90 cm.

Plate III: Early stage III undergoing degeneration (a) by an influx of elements, possibly phagocytes, which absorb the nuclear stain, haematoxylin; (b) stage II and (c) remains of degenerated oocyte. Length of fish is 72 cm.

Plate IV: Oocyte in the halfway point of degeneration. Note that the chorion (c) or zona radiata is still intact. Length of fish is 72 cm.

Plate V: Further degeneration of stage III oocyte. Length of fish is 80 cm.

Plate VI: (a) oocytes at the end of degeneration and reabsorption; (b) oocyte at beginning stage III, the new generation of oocytes. Length of fish is 68 cm.

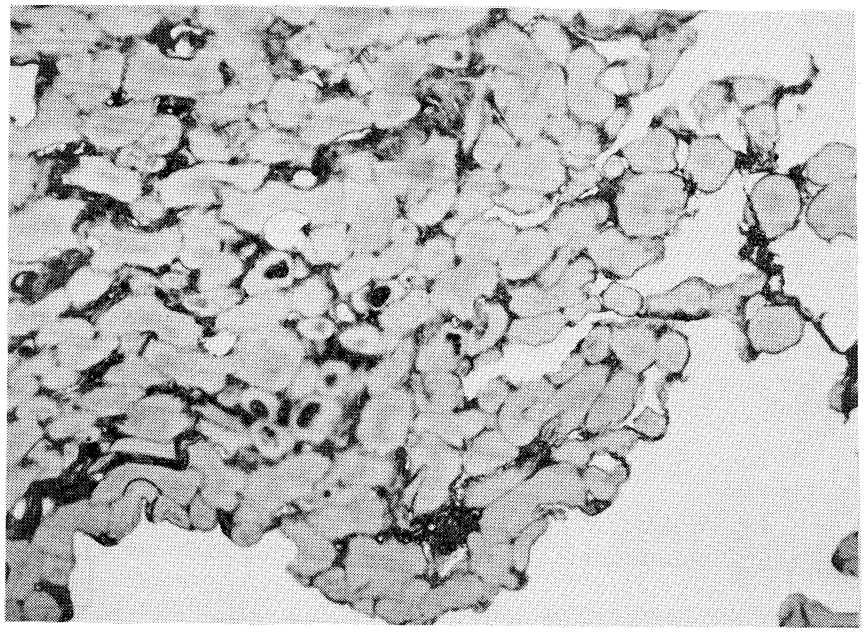


PLATE I

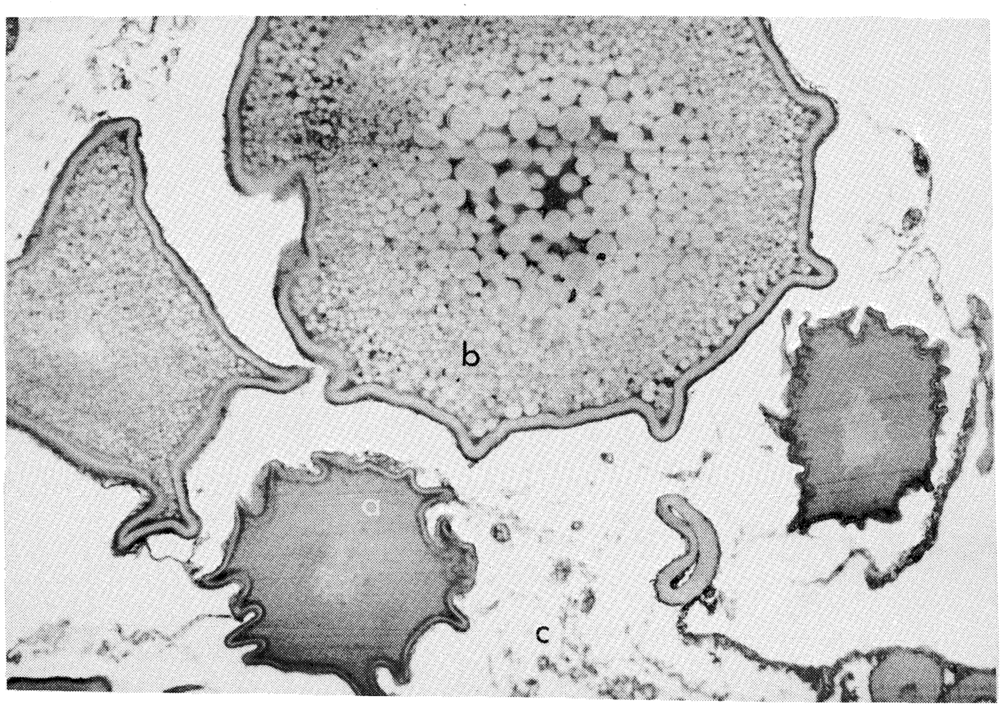
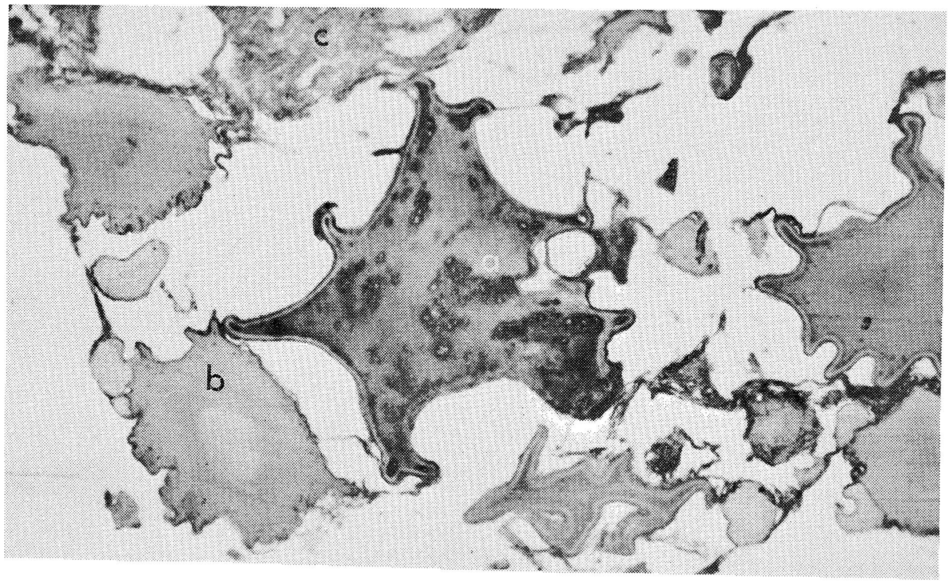


PLATE II



PLTAE III

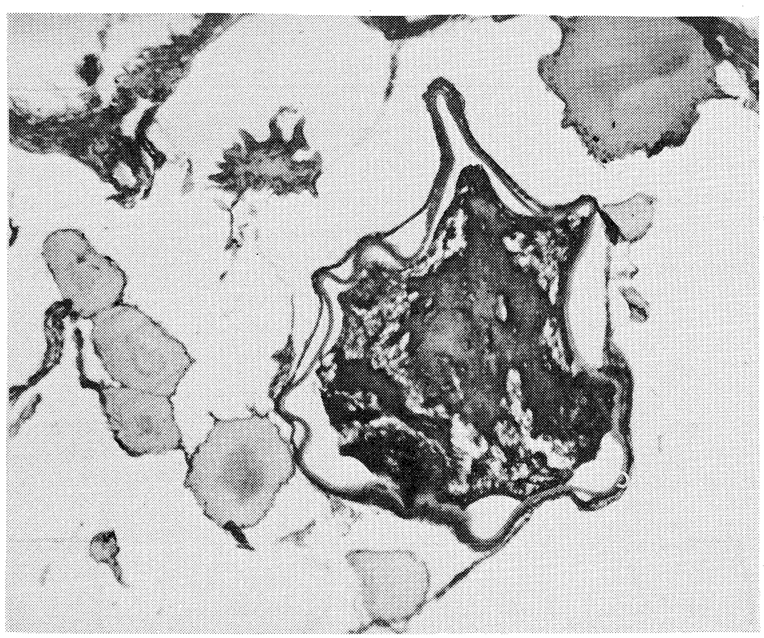


PLATE IV

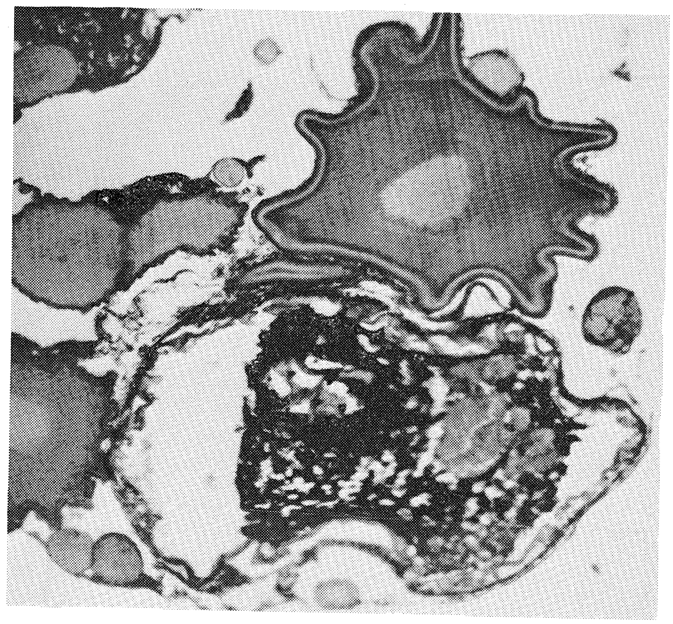


PLATE V

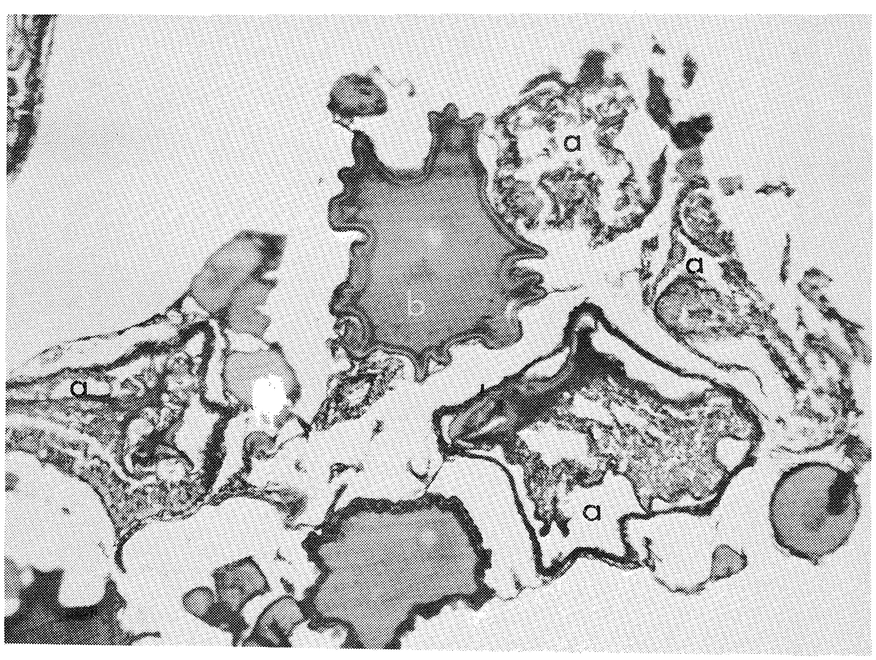


PLATE VI