ANATOMY

HISTOLOGY OF THE CORPORA LUTEA IN BLUE AND FIN WHALES OVARIES

BY

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Introduction.

One of the most important questions with which whale research is concerned is the problem of deciding the absolute age of a whale. MACKINTOSH and WHEELER (1929), WHEELER (1930) and LAURIE (1937) showed that the total number of old corpora lutea in both ovaries of a whale might be a clue, provided that the average number of ovulations in one year is known, as the old corpora lutea or corpora albicantia remain visible in the ovaries for a considerable time, probably for the rest of the whales' life. Now, the average number of ovulations annually can only be estimated very roughly and there is probably a great individual variation, depending among other things on the time of the year each individual has its first ovulation and the time of the year at which the animal becomes pregnant.

PETERS (1939) thought it possible to decide whether an old corpus luteum was one of pregnancy or one of ovulation only. The two kinds of old corpora lutea, according to this author, differed in macroscopical texture, colour, in amount of connective tissue, etc. If this is true, it would be possible to decide the number of corpora lutea of pregnancy in both ovaries of a whale, that is the number of pregnancies the animal has had. This idea induced us to study the histology of the corpora lutea and corpora albicantia in detail.

Materials and methods.

The ovaries of blue and fin whales (Balaenoptera musculus L. and Bal. physalus L.) were collected on board the Dutch floating factory "Willem Barendsz" during the Antarctic whaling seasons 1946 — 1949 by the biologists Dr E. J. SLIJPER, Dr W. VERVOORT, Mr W. G. BRAAMS and the author. The corpora lutea were weighed, measured and then slices were preserved in formalin 12 %. Small pieces of fresh material were also fixed in Helly and afterwards chromated in a mixture of chromalum and potassiumbichromate. Paraffin sections of Helly fixed material were stained for mitochondria according to ALTMANN, whereas paraffin sections of the formol fixed material were stained with haemalum-eosin, haematoxylin-picrofuchsin (VAN GIESON), orcein, etc. The freezing microtome sections were treated with fat stains (Sudan III; Sudan Black, etc.).

The corpus luteum of pregnancy.

The corpus luteum of pregnancy is a conspicuous spherical body with a mean diameter of 20 cm and a mean weight of 2600 g in blue whales (maximum and minimum weight among 50 corpora lutea 7500 and 800 gram resp.) and a mean diameter of 14.3 cm and a mean weight of 930 gram in fin whales (with a maximum and minimum weight among 25 corpora lutea of 2800 and 400 gram respectively). It protudes far out of the ovary often connected with the latter only by a narrow stalk or hilus. The place where the follicle burst is indicated by a dimple with a small pit in the centre (MACKINTOSH and WHEELER). The outer layer of the yellow body consists of connective tissue from which centripetal strands penetrate into the pale yellowish brown luteal tissue. The centre often contains a cavity filled with a liquor and loose connective tissue from which centrifugal strands radiate.

The luteal tissue itself is mainly made up of two kinds of cells which according to their probable origin will be named granulosa and theca lutein cells (fig. 1).

The granulosa lutein cells are large oval cells (mean size 50 by 30 micra) with an eccentric nucleus (mean diameter 10 micra). The cytoplasm contains mostly in its centre a cloud of numerous small fuchsinophile granules and furthermore many long threadlike or shorter clavate mitochondria.

The granulosa lutein cells lie in radial strands or groups, surrounded by the theca lutein cells.

In the course of pregnancy some cytological changes occur which are probably connected with a functional change. Nothing is known however of the functional activity of whales' corpora lutea in the different months of pregnancy. MACKINTOSH and WHEELER report a slight decrease in weight of the corpora lutea during the later months of pregnancy but I do not think this is significant. If there is a decrease it can only be very slight and no conclusion can be drawn from it as to the functional activity of the gland. JACOBSEN (1941) determined the hormone content of blue whale corpora lutea but he did not mention the length of the foetuses. On the other hand the cytological pictures are not always reliable if one takes account of the difficult conditions under which the material is fixed and of the long time which often elapses between the death of the animal and the fixation of the gland.

MACKINTOSH and WHEELER have noted that the granulosa lutein cells of young corpora lutea of whales contain many vacuoles. The same has been observed by HARRISON (1949) in the corpora lutea of the Ca'aing whale Globiocephala melaena. In our material I found one corpus luteum in which the large luteal cells were highly vacuolated. In this case no foetus could be found in the rather swollen uterus. Either the foetus was so small that it was overlooked or the corpus luteum was one of ovulation. The animal concerned was a blue whale caught on December 15th. The corpus luteum weighed 4250 grams, that is well above the average. In the other ante-partum corpora lutea only small vacuoles were found or vacuoles were entirely absent. The small acidophil granules stained slightly with Sudan Black but not with fat stains like Sudan III or scarlet red.

The cells of the interstitial tissue are supposed to be of thecal origin. HARRISON describes how thecal cells invade the granulosa tissue from the penetrating bloodvessels together with other adventitial cells and fibroblasts. They are mostly spindle-shaped and often contain clavate or clublike mitochondria. No collagenous nor elastic fibres are formed in this interstitial tissue, but the granulosa luteal cells become embedded in a network of reticulin fibres.

In the interstitial tissue immediately adjoining the granulosa lutein cells — probably in the intercellular spaces — small lipid droplets are often found; these droplets are birefringent, loosing their birefringence on heating up to approximately 60° C and regaining it when cooled. They show a rather weak SCHULTZE-LIEBERMANN reaction. It is uncertain whether these droplets of cholesterol have anything to do with the production of the steroid hormone(s) in the corpora lutea. No correlation was found between the amount of cholesterol droplets and the age of the corpora lutea (see CLAESSON and HILLARP 1947).

The bloodvessels are inconspicuous if compared with the bloodvessels in the ovarian stroma. The arterioles, venules and capillaries are unusually long. Nevertheless I cannot agree with the supposition put forward by DEMPSEY and WISLOCKI (1941) who investigated the corpus luteum of the humpback whale Megaptera nodosa, that the greater length of the capillaries by hampering the bloodflow in the gland would induce the central cavity. If this were true, all the corpora lutea of blue and fin whales, being much larger than those of the humpbacks, would have a central cavity, whereas only half the corpora lutea we investigated had any cavity at all.

The regression of the corpus luteum.

Immediately after parturition the corpus luteum begins to degenerate. No signs of regression have been found in corpora lutea of whales with large foetuses. In the corpora lutea of the lactating whales caught, degeneration had progressed so far already that one could speak of a corpus albicans: from the granulosa lutein cells only fat droplets remained, the theca lutein cells were already quite strongly collagenized. Later regression follows at a much slower speed, taking probably 3 or 4 years before degeneration is completed.

The two kinds of lutein cells undergo a degeneration very different from each other.

The granulosa lutein cells become vacuolated, they obtain a "fumy" appearance. The vacuoles contain a fatty (sudanophile) substance. In the course of the degeneration the light yellow colour of the droplets changes into a yellowish brown; the substance can still be coloured with Sudan Black then but no longer with Sudan III or scarlet red. From some remaining capillaries there arise in the mean time endothelial networks in which many macrophages and lymphocytes appear. These macrophages take up the brown granules and after the disappearance of the networks the macrophages lay scattered between the large bloodvessels.

The theca lutein cells dedifferentiate already at the end of pregnancy and attain a fibroblastlike appearance whereafter they begin to form collagenous fibres. After the degeneration of the granulosa lutein cells they form the bulk of the remaining, slightly collagenous, yellowish brown "luteal tissue". This luteal tissue diminishes and at the end of the regression of the corpus luteum no remains of it can be found except for the macrophages mentioned above.

The connective tissue of an old corpus albicans is mainly derived from two sources: the connective tissue already present in the active corpus luteum and the adventitial cells accompanying the bloodvessels. Among the first are the tunica albuginea on the outside and the connective tissue nucleus or the tissue surrounding the central cavity on the inside. Both contain only few elastic fibres but many collagenous fibres. The connective tissue arising from the adventitial cells contains many elastic fibres.

The collagenous tissue does not play an important part in the regression of the corpus luteum. It is gradually reduced in size and only a few narrow strands of collagenous tissue remain between the bloodvessels. (fig. 2.) The tunica albuginea becomes a part of the ovarian stroma as the corpus albicans sinks beneath the general surface of the ovary.

The capillaries disappear for the greater part but a few of the larger ones remain. They become surrounded by smooth muscle cells and adventitial cells. DAWSON (1946) finds for the corpus luteum of the cat that the adventitial cells play a big part in the postpartum induration. He describes how these adventitial cells penetrate from their loci into the luteal tissue, replacing the old luteal cells and forming a rather faint collagenous stroma. In whales this role is played by the theca lutein cells whereas the adventitial cells remain all around the bloodvessels. In their further development the small bloodvessels acquire some resem-

E. W. VAN LENNEP: *Histology of the corpora lutea in blue and fin whales ovaries.*

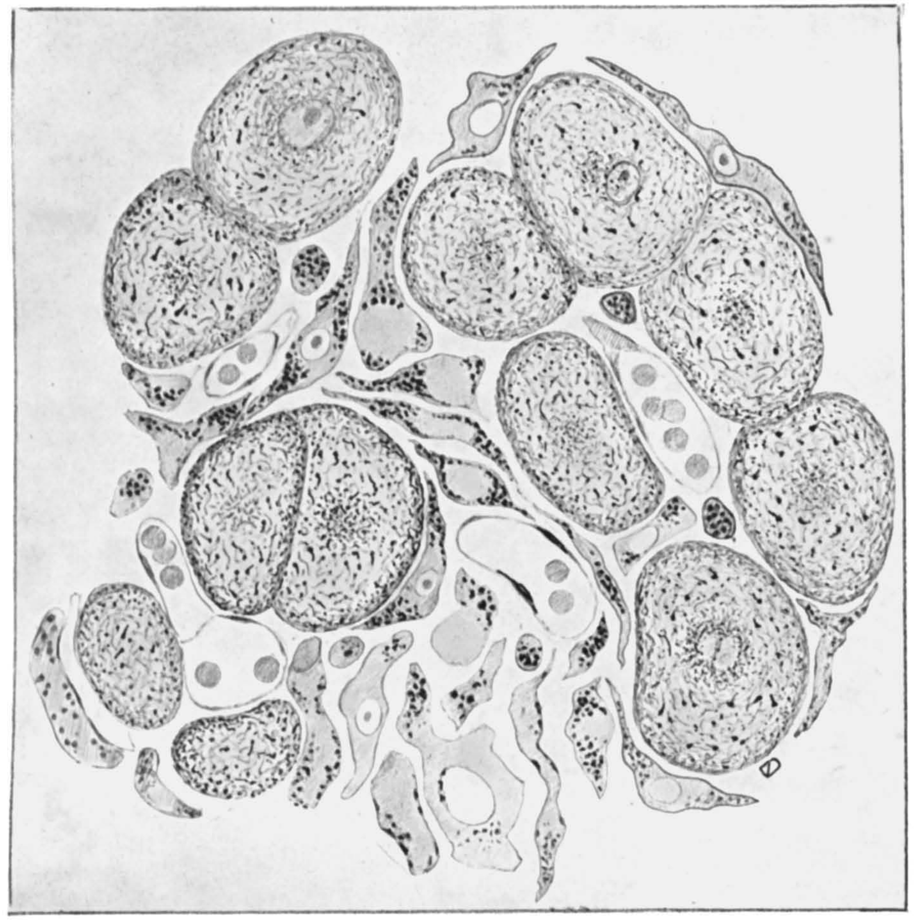

Fig. 1

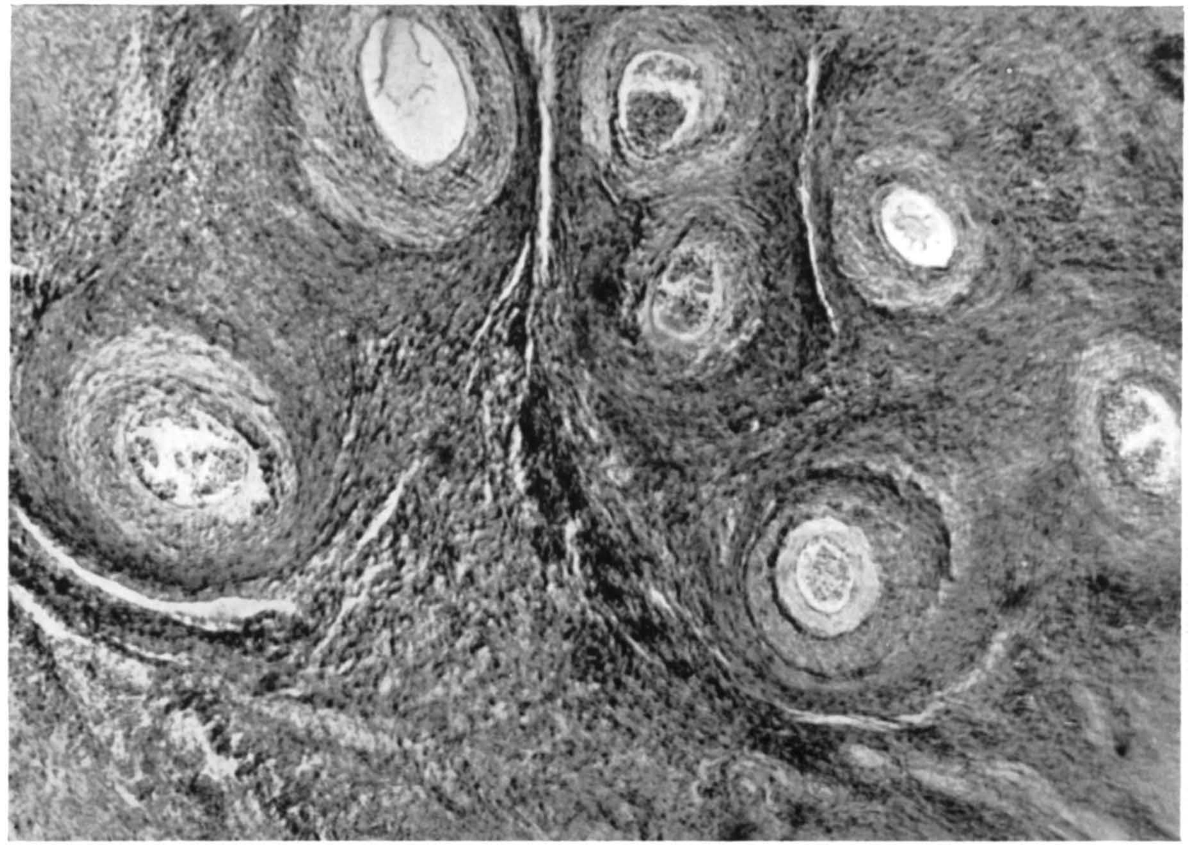

Fig. 2

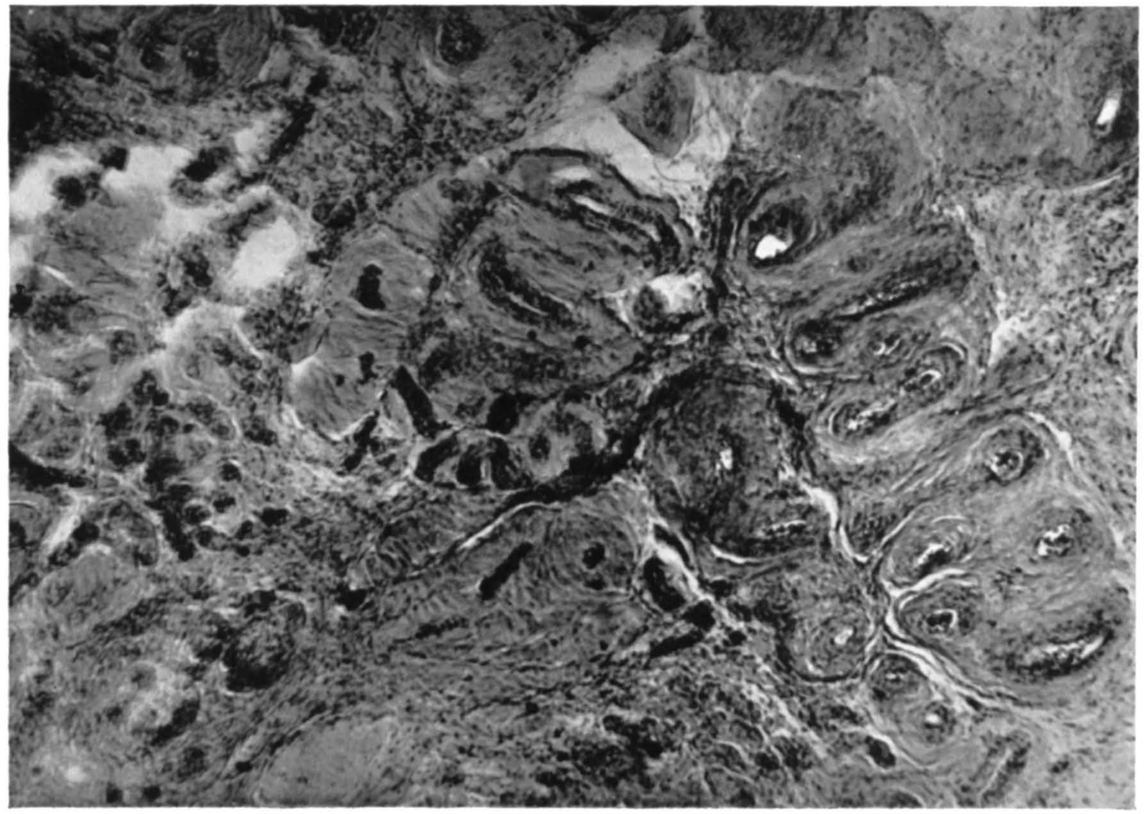

Fig. 3

Fig. 4

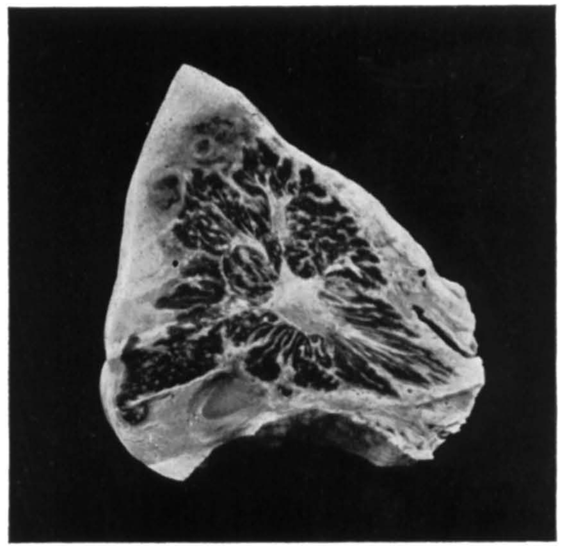

Fig. 5

EXPLANATION

Fig. 1. Balaenoptera physalus. Corpus luteum graviditatis. Large granulosa lutein cells with numerous threadlike or clavate mitochondria. Smaller spindleshaped or polymorphous theca lutein cells with more granular mitochondria. Note the large intercellular space. HELLY-ALTMANN. 600 ×.

Fig. 2. Balaenoptera physalus. Corpus albicans. Between the enlarged bloodvessels only narrow strands of connective tissue are left. Haemalum-eosin. 35 ×.

Fig. 3. Balaenoptera physalus. Corpus albicans. The twisted bloodvessels are surrounded by strands of luteal tissue containing numerous macrophages. Haemalum-eosin 35 ×.

Fig. 4. Balaenoptera musculus. Cross-section of a corpus albicans with a small central cavity and radial connective tissue strands. Natural size.

Fig. 5. Balaenoptera musculus. Cross-section of a corpus albicans of the branched type. To the left a few small follicles. Natural size.

blance to arterio-venous anastomoses which have been found in the ovaries of other mammals (MATHIS and EGLITIS 1936, WATZKA 1936 a.o.). They become twisted corkscrewlike (fig. 3), which development stands probably in connection with the reduction in size of the corpus albicans and the original length of the bloodvessel; the lumen is narrow and on the inside of the membrana elastica interna there is often a thick layer of smooth muscle cells, comparable with the "Gefäss-sperren" which WATZKA described.

The tunica media consists of a few muscle cells and collagenous tissue. In the first stages of development of the bloodvessels the tunica media is the most dominant of the three layers but in the end the external layer (membrana elastica externa) becomes far more important, so that a fully regressed corpus albicans consists almost only of elastic tissue.

Though it could not be proved owing to technical difficulties it is probable that the bloodvessels described are at least for a part true arterio-venous anastomoses as only very few capillaries remain in an old corpus albicans.

Discussion.

From the results of the histological study of the old corpora lutea no indication can be obtained that the old corpora lutea of pregnancy follow in their regression a pattern different from that of the corpora lutea of ovulation. The differences in texture PETERS described were certainly only differences in age of the corpora albicantia.

Yet another difference can be seen in young corpora albicantia: the trabeculae of connective tissue strands run in some corpora albicantia straight from periphery to centre (fig. 4); in others they are branched with often numerous ramifications (fig. 5). Most, if not all, corpora albicantia of the first type are probably derived from corpora lutea of ovulation as only young corpora lutea of pregnancy have been found to possess the same structure and may therefore be supposed to become corpora albicantia of the second (branched) type. This does not necessarily mean that all corpora albicantia of the branched type have been corpora lutea of pregnancy as corpora lutea of ovulation may very well be of a complicated structure but I had no opportunity to study corpora lutea of ovulation as the oestrous cycle takes place during the southern winter outside the season of antarctic pelagic whaling.

The mean diameter of a corpus albicans might give us an indication of its probable origin. According to MACKINTOSH and WHEELER the average diameter of corpora lutea of ovulation from blue and fin whales are 11 and 8 cm respectively and of corpora lutea of pregnancy 14 and 12 cm respectively. As corpora lutea of pregnancy contain more connective tissue than corpora lutea of ovulation the latter can be expected to be smaller as a rule than corpora lutea of pregnancy in the same stage of regression. Meanwhile the sizes of corpora lutea of ovulation and of pregnancy overlap each other, so that the size of the corpora albicantia cannot be decisive.

The fact that notwithstanding careful sectioning of hundreds of ovaries no corpora albicantia smaller than $1\frac{1}{2}$ cm diameter have been found and that these small corpora albicantia consist practically only of the thick elastic walls of bloodvessels makes it highly probable that the corpora albicantia never completely disappear during a whale's lifetime.

The same pattern of regression of corpora lutea as described above was found in the ovary of a porpoise (Phocaena phocaena L.). COMRIE and ADAM (1938) described some corpora lutea of the false killer whale Pseudorca crassidens but they give no histological details.

A discussion of the probable average annual number of ovulations and of the rate of regression of the corpora lutea will be given in a later paper.

Summary.

1. A histological description has been given of the corpora lutea of pregnancy of blue and fin whales.
2. A detailed account is given of the process of regression of the corpora lutea. During the regression the luteal tissue disappears and the lutein pigment is taken up by macrophages. An old corpus albicans consists solely of the thick elastic walls of the twisted bloodvessels.
3. It is highly probable that old corpora albicantia remain visible as tough lumps, 1 to 2 cm in size, during the whole life of a blue or a fin whale.
4. Two possible means of distinguishing between old corpora lutea of pregnancy and old corpora lutea of ovulation are suggested.

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