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HISTOLOGICAL STUDIES ON THE GONADS OF THE FOWL.*

II.—THE HISTOGENESIS OF THE SO-CALLED "LUTEAL" CELLS IN THE OVARY.

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WITH ONE PLATE.

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I. Introduction.

The presence of clear epithelial cells in the theca interna of the ovarian follicles of the fowl and other birds has been noted by several observers (Waldeyer, His, Poll, Sonnenbrodt, etc.), most of whom homologise this tissue with the interstitial cells of the mammalian ovary. The development of the islets was first worked out in full by Firket, who describes their origin from the medullary cords (sex cords of the first proliferation), and his results have recently been confirmed by Nonidez. The name "luteal" cells was first applied to the tissue by Pearl and Boring who state that in discharged and atretic follicles the islets multiply and migrate into the cavities

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of the follicles, and there give rise to a mass of vacuolated cells containing yellow pigment which, in the opinion of these workers, is homologous with the corpus luteum of mammals. Islets of the so-called “luteal” cells also occur in the gonads of late male embryos and young male chicks, and have been shown by Nonidez to be derived from sex cords and young tubules. They are absent, however, in the functional testis of the adult bird. General interest was first attracted to the “luteal” cells by the discovery by Boring and Morgan of considerable quantities of this tissue in the testes of adult cocks belonging to the hen-feathered breeds such as the Sebright bantam and Campine. According to Morgan, castration of such hen-feathered males effects the same result as spaying produces in the normal hen, viz., the assumption of well-developed typical male plumage. Since “luteal” cells are apparently the only gland-like elements common to both the testis of the hen-feathered cock and the ovary of the normal hen, it is concluded that it is this tissue which is responsible for the exhibition of female plumage. The recent work of Pease, however, has thrown doubt on this attractive hypothesis. This author states that “wherever material was taken from immature birds, or from birds where spermatogenesis was inactive or only beginning, “luteal” cells were always found not only in the hen-feathered material but also in the control material from normal birds,” and concludes that the presence of “luteal” cells in the testis depends on the state of spermatogenesis and is not associated with the type of plumage.

In view of the conflicting opinions concerning the nature and function of the “luteal” cells, it seemed desirable to undertake a reinvestigation of the subject, and the results of the first part of the study are embodied in the present communication.


The material employed consisted of the ovaries of twenty-two female embryos of the first generation of a Rhode Island Red x Light Sussex cross which were taken at the seventh, eleventh, fifteenth, seventeenth, nineteenth, and twentieth days of incubation, of thirteen young pullets of the same cross and of the following ages: one day, nineteen days, and two, three,
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and three and a half months; of eleven normal adult birds of various breeds, and of the gonads of the eight fowls showing different stages in sex reversal, which formed the subject of the first paper of this series. The seventh and eleventh day embryos were fixed whole, but in the other cases the ovaries were removed immediately after death and fixed in one or more of the following ways: by Da Fano’s silver impregnation method; in Flemming’s solution less acetic acid, followed by seven days’ post-chroming; in Allen’s chromic modification of Bouin’s fluid at 35°C.; and, for some of the post-embryonic material, the Mann-Kopsch osmic acid technique was employed. Regaud’s bichromate formalin was also tried but was not found to give very satisfactory results. Most of the sections were stained with Heidenhain’s iron haematoxylin, but Benda’s alizarin-crystal violet method and, for Da Fano and Mann-Kopsch preparations, safranin and acid fuchs in were also used.


Before beginning an account of the development of the islets of “luteal” cells, a brief description will be given of the embryology of the ovary as a whole.

According to Firket, the anlagen of the gonads appear at the end of the second day of incubation as bilateral thickenings of the celomic epithelium in the angles between the mesentery and the mesonephridia; the primary germ cells, which are formed early in embryonic life, migrate into these thickened areas. Owing to the increase in the subepithelial mesenchyme, the anlagen soon assume the form of ridges—the germinal ridges. Sex up to this point is indistinguishable. During the sixth day, however, the germinal epithelium of the ridges gives rise to buds of cells which proliferate rapidly to form epithelial cords permeating the subjacent connective tissue and known as the medullary cords. Beyond this point the gonad begins to exhibit the structural features of either ovary or testis, and the sex of the embryo can be recognised. In the male the medullary cords give rise to the seminiferous tubules, but in the female abort, together with the primary germ cells which they contain and are replaced by a second proliferation—the cortical cords. The latter begin to form at the eleventh
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day, and give rise to the definitive oocytes derived, according to Firket, from peritoneal cells, and according to Swift from the primary germ cells remaining in the epithelium after the formation of the medullary cords.

Examination of the gonads at the seventh day of incubation reveals no trace of developing "luteal" tissue, but by the eleventh day, sections show several groups of these cells in course of formation. As Firket and others have shown, most of the medullary cords abort—cavities appear in their centres and enlarge until the medullary region is reduced to a mere network of cellular strands. In the eleventh day embryos the lacunæ are beginning to appear and, in the more central cords, are already of considerable size; the transformation of medullary cords into "luteal" cells is taking place throughout the ovary but is most advanced between, and in the area just distal to the large lacunæ, into which groups of "luteal" cells often project. The first stage in the transition is the increase in number of the mitochondria of the epithelial cells which, in Da Fano material, causes the developing islets to appear more deeply impregnated than the surrounding tissue, so that under low power the better developed groups are seen as conspicuous dark brown patches. The mitochondria are in the form of stout filaments, usually short but of variable length. The nucleus of the transforming sex-cord cells also undergoes certain changes; it diminishes in size, and, in unstained Da Fano preparations, assumes a reddish-brown colouration, instead of appearing as a more or less clear space as in the ordinary sex cord cells. In Allen material the most advanced "luteal" cells show a distinct outline and have an irregularly vacuolated cytoplasm which has not, however, the startlingly clear appearance so characteristic of the tissue in older gonads; the nucleus stains more deeply with iron hæmatoxylin than do those of the surrounding cells, and contain several large chromatic or nucleolar granules. Primary gonocytes are, as Firket and Nonidez affirm, occasionally found in an islet.

In the ovaries of fifteenth day embryos the number of islets is greatly increased. The lacunæ are much more numerous and the proximal half of the medullary region is reduced to
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a mere network studded with small groups of "luteal" cells, some of which are disintegrating. The larger and more deeply impregnated islets are now situated in the distal half of the medulla; the groups are enclosed in a basement membrane which is better developed that in the original sex cord. In material fixed in Flemming's solution and stained with iron haematoxylin, the "luteal" cells are seen to contain numerous mitochondria while the ground substance of the cytoplasm is vacuolated (l.c., fig. 1). In the earlier formed groups most of the mitochondria have lost their filamentous form and appear as granules of rather variable size.

By the seventeenth day almost all the medullary cords are either canalicolised or transformed into "luteal" cells, whilst the cortical cords have greatly enlarged and have almost obliterated the broad band of connective tissue (ovarian albuginea) intervening between the germinal epithelium and the medullary cords. The cytological character of the "luteal" cells remains unchanged.

In the nineteenth day embryos the "luteal" cells have begun to assume the characteristic appearance met with in the adult bird. In material fixed in Flemming's solution, the cytoplasm in the majority of the islets is occupied by the regular spherical vacuoles which are much more marked than at the fifteenth day, and the islets are therefore rendered more conspicuous than in the younger gonads. Firket, in his account of the interstitial cells of an eighteenth day embryo, describes two types, one of which corresponds to the so-called "luteal" cells, the other being different in histological character, localisation, and origin. According to this author, the second form occurs as groups of spindle-shaped, fat-laden cells which are found at intervals along the line of division between the cortical and medullary cords and which are derived from connective tissue cells. Nonidez was unable to confirm the occurrence of this second form. They are well seen, however, in my material, in a nineteenth day ovary fixed in Flemming's solution, where they occur in groups of five or more cells enclosed by a limiting membrane (fig. 2). The shape of the cells is variable, but, as Firket states, is usually more or less fusiform; the nuclei, which are oval or spherical, contain in
addition to the nucleolus, numerous granules, and are considerably larger and less deeply staining than those of the "luteal" cells; the cytoplasm is rather dense and contains fat globules of various sizes. So far the present account agrees with that of Firket. The writer is, however, unable to confirm the former's statement that the cells originate from connective tissue elements and regards them as being derived from undifferentiated sex-cord cells (i.e. cells which have not developed into secondary gonocytes) at the apex of the cortical cords. The evidence in support of this view is as follows: (1) In the ovary of the seventeenth day embryo, in which no islets of the second form were found, the cortical cords had not for the most part come in contact with the medullary cords. Some, however, had done so, and in such cases it was noticed that the central ends were composed of undifferentiated cells containing fat globules and presenting exactly the same histological characters as the cells above mentioned. (2) Groups of "interstitial" cells are found in continuity with the cortical cords and enclosed in the same membrane. (3) Fat globules are numerous in most of the undifferentiated epithelial cells which are situated in the central ends of the cords. (4) The character of the nucleus resembles that of the undifferentiated sex-cord cells rather than of the connective tissue elements. In the day-old chick the "interstitial" cells are present in greater numbers and larger groups, and are most conspicuous in Mann-Kopsch preparations in which they are very clearly differentiated from the "luteal" cells. The cytoplasm of the latter, as in the adult bird, is so deeply impregnated with the osmic acid that, under low power, the islets appear as dense, black blotches (fig. 3) which, though rendered considerably paler, are readily distinguishable even after prolonged immersion in turpentine. The small spherical vacuoles with which the cytoplasm is honeycombed, remain clear, however. The "interstitial" cells, on the other hand, are conspicuous owing to the globules of osmicated fat with which they are crowded, and the ground substance of the cytoplasm is yellowish as in neighbouring cells.

By the nineteenth day after hatching the medullary cords are no longer recognisable as such, but in the proximal region
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are represented by a network of fibrous and cellular strands, and in the distal portion by large lacunæ lined by epithelial cells of fairly normal appearance; the cortical region is greatly thickened, partly by the enlargement of the oocytes, and partly by the increase in amount of connective tissue stroma. All the oocytes have now a membrana granulosa but the theca interna and externa are not yet laid down. Owing to the increased volume of the ovary as a whole, and to the absorption of the medullary cords, the number of "luteal" cells per unit volume is somewhat reduced. The islets are situated on the hilar border of the cortex and in the peripheral remnants of the medullary cords, but as yet none are found between the closely packed oocytes. The histological character of the "luteal" cells has altered slightly and, in sections from material fixed in Flemming's solution, the cytoplasmic vacuoles are seen to be larger and the nucleus often slightly crenated; in Allen preparations the islets have the characteristically clear appearance of the adult ovary.

In the two months' chick a small proportion of islets are beginning to appear in the cortex itself in regions where the larger oocytes are situated. The cells penetrate into the cortex, not by active movement of their own, but, as Firket has stated, by the enlargement of the oocytes. In the areas of larger oocytes referred to above most of the stroma and also the peripheral remnants of the medullary cords (fig. 4) have, probably in response to mechanical forces only, become concentrated round the enlarging oocytes to form a theca interna, in which the islets which these tissues already contained are also incorporated.

In the ovary of the three and a half months' pullet it is found that the majority of the "luteal" cells are now incorporated in the ovarian follicles, and the embryonic development of the islets is now completed.

4. The Formation of "Luteal" Cells in the Adult Ovary.

(1) In the Normal Ovary.—If a section of an adult ovary is compared with one of the ovary of a three weeks' chick, it is seen that the number of "luteal" cells per unit area is approximately the same in both. When the great increase
in volume of the ovary is borne in mind, it is evident that
the number of original islets must have considerably increased.
This multiplication of the "luteal" cells has been noted by
several authors, e.g. Sonnenbrodt, Firket, and Nonidez. The
last mentioned states that he has been unable to find any trace
of karyokinesisis in this tissue and thinks it improbable that
it ever occurs—an opinion with which the present writer is
in entire agreement. Throughout the study, not a single
mitotic figure was encountered. It was found that the
multiplication is due to a neoformation from certain cell
elements in the follicular thecae and general stroma. Although
the process occurs to a certain extent in the normal follicles
and the stroma, it is much more striking and more readily
followed in the thecae of atretic oocytes, in which it will
accordingly be described. As the resorbing oocyte shrinks,
the cells of the theca interna become loosened and sometimes
assume a columnar arrangement, the columns being at right
angles to the surface of the ovary. The cells destined to
give rise to new "luteal" tissue occur singly or more commonly
in more or less diffuse groups; they have a rather large, clear,
spherical nucleus containing two or more nucleolar particles
and several smaller chromatin granules; the cytoplasm is
lightly staining, has a somewhat vague polyhedral or slightly
fusiform outline, and contains small rod-shaped mitochondria.
As in the embryo, the first stage in the formation of "luteal"
cells is an increase in the number of mitochondria, together
with an enlargement of the cytoplasm and a greater definition
of outline. The nucleus then becomes slightly smaller and
more chromaphil; the majority of the mitochondria assume
a granular form; cytoplasmic vacuoles begin to appear which,
owing to the large number of the mitochondria, are at first not
readily distinguishable. The granular mitochondria enlarge,
but show great variation in size; they decrease in number
and the vacuoles become more conspicuous—it is noticeable
that, roughly speaking, increase in number and size of the
vacuoles is proportional to the decrease in amount of mito-
chondrial material. A few rod-like mitochondria usually persist
and take up an intervacuolar position, which emphasises the
boundaries of the vacuoles so as to produce the conspicuous
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honeycombed appearance so characteristic of the cytoplasm of the fully formed "luteal" cell. The granular mitochondria finally appear as a few large, irregular blocks lying in the protoplasm.

It would appear at first sight that the cells from which "luteal" tissue is derived, are, as Firket believed, connective tissue elements, but this is probably not the case. Examination of the ovary of the chick shows that the follicular theca which is forming round the enlarging oöcyte includes not only connective tissue and "luteal" islets, but also, as stated above, the peripheral ends of the large lacunæ which represent the remains of the medullary cords. These lacunæ in the distal regions of the ovary are, for the most part, still lined by fairly well-marked cubical epithelium; as the oöcyte grows, the walls of the lacunæ are approximated and form split cords of epithelial cells in the theca interna. This process was very clearly seen in sections of a two months' ovary fixed by the Mann-Kopsch method, which were stained with acid fuchsin (m.c. 1 and 2, fig. 4). In later life these cords lose their distinctness owing to the increased density of the connective tissue, and the cells become isolated or arranged in scattered groups. If the forerunners of the new "luteal" cell islets in the adult are compared with the medullary cord cells in the developing follicles of the chick ovary, it is seen that the cytological character is identical in the two cases. Similar scattered groups of undifferentiated medullary cord cells have been described and figured by Nonidez in the cortical stroma of the ovary of an eight-day chick. These are evidently incorporated in the stroma by the thickening of the cortex in a manner similar to that which has just been described in the case of older ovaries. Nonidez also expresses the opinion that it is from such epithelial elements that "luteal" cells are formed in the gonads of adult individuals, but does not describe the process by which this transformation takes place.

The cytological structure of the fully formed "luteal" cell is as follows: the nucleus is usually spherical, but may be slightly crenated; in Allen preparations it is seen to contain one or more nucleolar particles and numerous small chromatin granules; it assumes a dark grey coloration after treatment
with the Mann-Kopsch technique, and in preparations made by Da Fano's method becomes dark reddish-brown in colour, and one or more black spots are seen—particles of nucleolar material. In material fixed in Flemming's solution the nucleus stains more deeply with iron hæmatoxylin than is the case in the surrounding stroma cells. The cytoplasm which, as stated above, is honeycombed with spherical vacuoles, varies greatly in appearance according to the fixative employed. If the material is preserved in picro-acetic fluids, the protoplasm stains very lightly, the vacuoles have a rather indefinite outline, and the cells are conspicuously clear in comparison with the surrounding tissue. In preparations made by the Mann-Kopsch method the protoplasm is often so deeply impregnated as to be quite black, which even under low power causes the "luteal" islets to stand out sharply against the greenish stroma the vacuoles are clear except in a few instances in which pale globules of osmicated fat can be detected. The Da Fano technique also causes the islets to contrast sharply with the surrounding tissue owing to the dark impregnation; the vacuoles are of very vague outline (fig. 5). With these two techniques the most deeply impregnated islets probably represent those most recently formed, in which the cytoplasm is crowded with mitochondrial granules. The Golgi apparatus could only be studied in the Da Fano material, and the structure is difficult to distinguish owing to the small size and deep impregnation of the cell. It appears to consist of a group of three or more granules concentrated round the archoplasm (g. fig. 5), and having a darkly impregnated periphery and a relatively light centre. The rod-like mitochondria and mitochondrial granules are best seen in material fixed in Flemming's solution less acetic acid, and stained by Benda's alizarin-crystal violet method (fig. 6). In such preparations the vacuoles are found to be regular in outline, and not uncommonly are seen to contain fat globules of a pale greenish coloration, which are very difficult to detect with other methods.

As the age of the islets increases degenerative changes begin to appear, the nucleus becomes more and more crenated; the cytoplasmic vacuoles enlarge and the boundaries of individual cells can no longer be made out; the mitochondrial
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material disappears and both nucleus and cytoplasm lose their capacity for deep impregnation. The ultimate fate of these cells could not be determined.

(2) In Cases of Sex Reversal.—The second mode of formation of “luteal” cells occurs only in cases of sex reversal and is really a repetition of the embryonic process.

In sex reversal the new germinal tissue which is going to give rise to seminiferous tubules is developed from a proliferation of sex cords from the germinal peritoneum covering the surface of the ovary. As the phenomenon has already been described elsewhere, only a brief outline will be given here. The germinal epithelium thickens considerably owing to the enlargement of the individual cells; the proximal ends of the cells form finger-like processes which penetrate the basement membrane; the latter finally breaks down altogether and buds of epithelial cells begin to grow inwards into the stroma, where they give rise to anastomosing cords of epithelial cells which may ultimately develop into tubules. The degree of differentiation of the cords and tubules is very variable in different regions of the same gonad. In some areas the cells of the cords and smaller tubules are principally primary spermatocytes and the large tubules are indistinguishable from those of a functional cock, while elsewhere the cords are composed of undifferentiated epithelial cells, and the tubules are lined by columnar epithelium showing no spermatogenetic stages. The degree of development attained by the neoplastic germinal tissue in the ovaries of different individuals is also very variable—in some are found tubules containing spermatozoa, whilst in others only undifferentiated sex cords are present. It is from such undifferentiated cords that “luteal” cells are formed (fig. 7). The cords are composed of polyhedral cells with a large, clear, spherical or oval nucleus containing a plasmosome and cytoplasm showing numerous mitochondria; the cell outlines are often vague; a distinct basement membrane bounds the cord. As in the case of the embryo and normal adult, the first stage in the development of “luteal” cells is represented by an increase in the number of mitochondria followed by the formation of numerous small vacuoles. The nucleus becomes reduced in size, is stained and impregnated
more deeply than in the neighbouring cells, and sometimes shows a crenated outline. The fully formed cell, in all its somewhat characteristic cytological features, is indistinguishable from those of the normal ovary. Often several adjacent cords undergo the same process, thus giving rise to enormous groups of "luteal" cells which are startlingly conspicuous in Allen preparations. In view of Nonidez' suggestion that these cells may be of a degenerative character, it is of interest to note that they are always far more numerous in areas in which the cords are completely undifferentiated and frequently aborting.

5. Discussion.

The foregoing account of the development of the islets of so-called "luteal" cells from the medullary cords of the embryo, agrees in all essentials with those of Firket and Nonidez. It would appear also, from the results obtained in the study of the adult ovary, that Nonidez is correct in his suggestion that the multiplication of this tissue in post-embryonic life takes place only at the expense of medullary cord cells included in the stroma and not, as Firket thought, by a transformation of mesenchymatous elements. On theoretical grounds Firket's view seems improbable, for, as Nonidez points out: "Estas células representan un tipo especifico y especializado dentro del ovario."

The two functions attributed to the "luteal" cells are (1) the elaboration of an internal secretion which is responsible for the inhibition of the male plumage, and (2) the formation of a corpus luteum in discharged and atretic follicles.

The former hypothesis receives support from Morgan's experimental work on hen-feathered cocks, to which reference has already been made, but a study of the mode of formation of the islets affords little evidence in its favour, and suggests that this tissue is of a degenerate character. Nonidez inclines to the opinion that the "luteal" cells are the result of fatty infiltration of the cords, and by means of frozen sections has demonstrated fat in the cytoplasmic vacuoles in the islets of the adult ovary. A more detailed cytological investigation supports this view. Up to a certain point the mode of development closely resembles that described by Dubreuil for
adipose cells in the connective tissue. In both the first stage is represented by a multiplication of the mitochondria, followed by the appearance of small fat vacuoles. Dubreuil describes a process of transformation of the mitochondria into lipid vacuoles. The former decrease in number and only those of a granular structure persist; they enlarge, often to a considerable size, and then apparently transform into similarly staining lipid vacuoles, which in turn appear to give rise to fat vacuoles. The formation of lipid vacuoles and their transformation into fat vacuoles was not seen in the “luteal” cells, but it seems probable that the mitochondria are concerned in the manufacture of fat globules since, as stated above, the decrease in the number of the former is roughly correlated with the increase in number and size of the latter. On the other hand, the transformation of rod-like mitochondria into a granular form, and their subsequent partial or complete disappearance from the cytoplasm appears to be a usual phenomenon in cell degeneration as described in the pancreatic cells in cases of phosphorus’ poisoning (Scott), or in the gastro-intestinal epithelial cells during vitamine deficiency or acute starvation (Miller). According to Scott these changes sometimes take place in a cell which is synchronously undergoing fatty infiltration; but, although a fusion of mitochondria to form lipid globules is described, there is no apparent relationship between the two processes. Degenerative changes in such cases are not, however, preceded by the noteworthy multiplication of mitochondria which takes place in the development of the “luteal” cells, and are therefore probably not equivalent to those involved in the formation of the latter tissue. Although in the earlier stages the formation of adipose tissue so strongly resembles that of the “luteal” cells, the later development is very different. To quote from Dubreuil: “Enfin la cellule tend vers son aspect définitif. Le centre est occupé par un gros globule de graisse. Autour de celui-ci régne une écorce protoplasmique d’une certaine épaisseur qui est criblée de petites vacuoles graisseuses.” At no stage of their existence do the “luteal” cells show this structure, and fusion of the vacuoles either does not occur at all or only does so to a negligible extent. Correlated with this, the nucleus
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always retains its central position and spherical shape, whereas
in the adipose cells it becomes flattened and forced to one
side by the formation of the large central fat globule. The
cytological character of the fully formed "luteal" cells there-
fore resembles that of secretory rather than adipose tissue.
The presence of a highly soluble fat in the vacuoles does
not necessarily indicate that the cells are not of a secretory
character, since the fatty substance might well have an
endocrine function. Such a view, however, seems improbable
in consideration of the fact that formation of "luteal" cells
is always associated with degenerative structures. In the
embryo they are developed from the aborting medullary cords;
in cases of sex reversal from undifferentiated sex cords of
which many are atrophic. In the normal adult they are
formed in greatest numbers in the thecae of small atretic
follicles, which suggests that the fatty material is in some
way derived from the resorbing oöyte. These facts definitely
indicate that the transformation of the epithelial cells into
"luteal" tissue is the result of decreased vitality, in which
case it is difficult to understand how this tissue can exercise
any active physiological function. That there is some
correlation between the presence of "luteal" tissue in the
gonad and the exhibition of female plumage seems probable,
but does not justify the assumption that the former bears any
causal relationship to the latter, since both may be the result
of a third factor. In view of Geoffrey Smith's work on the
parasitism of Sacculina upon Inachus, it is possible to explain
such a correlation in terms of fat metabolism. Smith found
that certain fatty substances are present in considerable
quantities in the blood of the female in order to supply
material for yolk-production. These substances form an
important part of the food of the Sacculina, which thus acts
upon the physiology of the female like a ripe ovary and causes
little change in the metabolism. In the male crab, only a
small amount of the fatty materials are present, of which
a minimal quantity is essential to the life of the individual.
In order to maintain this minimum in the blood, and at the
same time supply the heavy demands of the Sacculina, the
metabolism of the parasitised male changes profoundly,
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and approximates that of the female. Associated with this metabolic change is the assumption of the female secondary sexual characters. It seems not wholly unlikely that such a metabolic control of the secondary sexual characters might also apply to the fowl and other oviparous animals with heavily yolked eggs. In order to explain the facts concerning castration and plumage, it would be necessary to assume that the gonad of either sex stimulates the production of lipoid materials in the blood, but that the testis does so to a considerably smaller extent than does the ovary. If the lipoid content of the blood is greater than a certain amount \(x\), the plumage will be of the female, and if less than \(x\), of the male type. In the normal female the amount would be above \(x\); ovariotomy would cause it to fall below, and the bird would become cock-feathered. In the normal male it would be below \(x\), and castration would cause a slight fall as perhaps expressed by the more luxuriant plumage of the capon. In the case of the hen-feathered Sebright and Campine cocks, the fat concentration would be slightly above \(x\); castration would cause it to fall below, and male plumage would be exhibited. If female plumage is produced by the higher fat content of the blood, it would not be surprising that fatty degeneration of inactive tissue, such as the medullary cord cells, on non-functional tubules should be most extensive in hen-feathered individuals.

The above hypothesis is tentatively put forward, since speculation unfounded on experimental data must be of small value. It is proposed, however, to put the theory to the test by making quantitative estimations of the lipoid content of the blood in the two sexes. In any case it is not untimely to raise a note of warning against the prevailing assumption that the gonads, and, in mammals, the interstitial cells in particular, are organs of internal secretion. As Hogben points out in his review “The Experimental Analysis of Sex”: “It is indeed imperative to recognise that the hypothesis of internal secretion, whether in relation to primary or secondary sexual differences is at present on its trial, for no investigation has conclusively produced by administration of the extracts made from the gonads of any animal, structural changes complementary to
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the disturbances produced by gonadectomy, or comparable in any way to the effects of transplantation of ovarian and testicular tissue. Until this has been accomplished it can only be legitimately concluded that the gonads influence the metabolic processes involved in sexual differentiation—primary or secondary—whether this influence is of an endocrine nature no crucial data at present exist to decide.”

The hypothesis put forward by Pearl and Boring, that the islets of “luteal” cells in the theca of discharged and large atretic follicles give rise to a corpus luteum, will receive only brief consideration here, as a further communication dealing with this aspect of the subject is in course of preparation. Pearl and Boring base their hypothesis on the appearance of enormous numbers of vacuolated cells in the walls of such follicles, which they homologise with those of the “luteal” islets. The present writer is unable to confirm this view, and finds that the cells are almost entirely the product of fatty degeneration of ordinary connective tissue cells; they have only a superficial resemblance to the islet tissue from which they differ markedly in minute cytological character. The process by which they are formed is readily distinguishable from that described for the development of new islets in the theca of the smaller atretic follicles. The yellow pigment upon which Pearl and Boring lay so much stress in their attempt to homologise the tissue occupying the discharged follicle in the fowl ovary with the mammalian corpus luteum, appears to be hæmatoidin which is derived from the numerous aborting blood vessels of the follicle. The vessels are invaded by wandering cells which ingest the erythrocytic debris; all stages in the intracellular formation of the yellow pigment can be followed. These cells synchronously or subsequently undergo infiltration with fat or lipoid, which produces the superficial resemblance to “luteal” tissue which caused them to be confused with the latter. The abortion of blood vessels is not entirely confined to atretic and discharged follicles, but may take place to a small extent in other parts, thus giving rise to small islets of vacuolated pigment containing cells.

From a morphological study alone, it is impossible to determine what is the true function of the “luteal” cells. They
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may play no part whatever in the economy of the ovary, or Nonidez may be correct in his suggestion that they have a trophic value for the young oöcyte. Their mode of formation seems, however, to point to the following conclusions:

1) That the cells are not of a secretory nature, but are the result of a rather peculiar form of fatty degeneration of sex-cord tissue.

2) That they bear no relation whatever to the mammalian corpus luteum. The usual view that the "luteal" cells are homologous with the interstitial cells of the mammalian ovary seems probable, since in many species of mammals the latter tissue contains large quantities of lipoid and fatty material. The origin and physiological significance of the interstitial cells is at present, however, the subject of so much diversity of opinion, that it is impossible to be certain on this point.


1) The so-called "luteal" cells of the ovary are derived from the medullary cords of the embryo, as previously shown by Firket and Nonidez. The process, which appears to be a peculiar form of fatty infiltration, begins about the eleventh day of incubation.

2) Multiplication of the cells occurs in post-embryonic life and, as suggested by Nonidez, appears to take place not by mitosis but by a neoformation at the expense of certain epithelial elements in the stroma and ovarian thecae, which are derived from the distal extremities of the medullary cords.

3) The fully formed "luteal" cell is seen to have a rather small spherical nucleus and vacuolated cytoplasm, which impregnates very deeply with the Mann-Kopsch and Da Fano techniques, and which contains blocks and granules of mitochondrial origin. With Benda's stain fat can sometimes be demonstrated in the vacuoles. Golgi bodies are situated round the archoplasm.

4) The formation of "luteal" cells in cases of sex reversal is found to be a repetition of the embryonic process. Sex cords are proliferated into the ovarian stroma and either give rise to seminiferous tubules or remain undifferentiated and...
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form typical "luteal" cells. In the latter case usually many of the neighbouring cords are degenerating.

(5) The mode of origin of the "luteal" tissue in the embryos, normal adult and cases of sex reversal, is difficult to reconcile with Morgan's theory of the endocrine activity of these cells in relation to plumage.

(6) In view of Geoffrey Smith's work on Inachus and Sacculina, it is suggested that the exhibition of hen-feathering may possibly be due to a high lipid content of the blood. On this assumption, the association of female plumage with the presence of "luteal" cells in the gonad would be explicable, since inactive tissue such as the medullary cord cells would be more prone to fatty degeneration in such hen-feathered individuals.

(7) It is thought that Pearl and Boring are mistaken in homologising the "luteal" islets with the cells of the mammalian corpus luteum, since the numerous vacuolated cells of discharged and large atretic follicles are found to be mainly the product of fatty degeneration of ordinary connective tissue elements, while the yellow pigment seems to be derived from aborting blood vessels. This pigment is regarded as hæmatoidin and is not found in the true "luteal" cells.

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7. References.

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8. Description of Plate.

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**Fig. 1.**—Section of ovary of fifteenth day embryo. (Fixation : Flemming's solution less acetic acid; stain : iron haematoxylin.) Drawing to show medullary cord in process of transformation into "luteal" cells (l.c.). Note the vacuolated cytoplasm and numerous mitochondria. On the right is seen an unaffected cord (m.c.), and on the left one which has become canalised.

**Fig. 2.**—Section of ovary of nineteenth day embryo (technique as above). Drawing to show group of Firket's second form of interstitial cells, situated at the junction of cortical (c.c.) and medullary cords. Note the fat globules in the "interstitial cells" and also in the undifferentiated cells of the cortical cords.

**Fig. 3.**—Section of ovary of day-old chick (fixed by the Mann-Kopsch method, unstained). Photograph showing the dense impregnation of the "luteal" cells. The cytoplasmic vacuoles appear as small clear spaces. × 787.5.

**Fig. 4.**—Section of ovary of two months' chick. (Fixation : Mann-Kopsch method; stain: acid fuchsin.) Photograph of developing follicle of young oocyte. Note the medullary cord cells (m.c. 1) already included in the follicle, and part of a large medullary lacuna (m.c. 2) which will eventually be incorporated. × 787.5.
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Fig. 5.—Section of ovary of adult bird. (Fixation: Da Fano's method; stain: safranin.) Drawing of "luteal" islet in the thèca of a small oöcyte. The Golgi bodies (g.), nucleolar particles, and vacuolated cytoplasm are well seen.

Fig. 6.—Same. (Fixation: Flemming's solution less acetic acid; stain: Benda.) Drawing of "luteal" cells showing the numerous mitochondria and irregular granules of mitochondrial origin in the cytoplasm.

Fig. 7.—Section of gonad in a case of sex reversal. (Fixation: Allen's solution; stain: iron hæmatoxylin.) Photograph showing three large groups of "luteal" cells. In the lower half of the figure are seen sex cords in course of transformation into "luteal" tissue (d.l.c.). Unaltered sex cords can also be distinguished among the stroma. × 337'5.
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