Some Degenerative Phenomena in *Drosophila* Ovaries

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**WITH FOUR PLATES**

**INTRODUCTION**

There are several reasons why it is of interest to examine the ultra-structure of degenerating cells. In the first place, processes of cellular degeneration may be involved in normal developmental processes. In the formation of the insect egg, for instance, a great deal of the ooplasm is derived from the substance of the nurse cells, which degenerate and eventually disappear as the oocyte reaches its final stage of growth. Again, the follicle cells, after secreting the chorion, degenerate and eventually disappear. As will be demonstrated in this paper, degenerating cytoplasm often exhibits some rather well-organized and elaborate structures which might have been taken, if one did not know of the cells in which they were found, to be organelles concerned with synthetic activities. A study of such materials suggests the dangers of too easy interpretation of some of the appearances seen in healthy cells.

The degeneration of the nurse cells, and the passage of material from them into the oocyte, in the normal development of the wild type *Drosophila* ovary will be considered in another paper. Three types of degenerative phenomena will be discussed here: (1) the degeneration of the follicle cells; (2) changes taking place in unfertilized eggs subsequent to laying; and (3) degenerative changes in the nurse cells of flies homozygous for the female-sterile gene *deep-orange*. Females homozygous for this gene can produce fertilizable eggs which die at an early stage of development unless fertilized by sperm carrying the normal allelomorph of the gene (Counce, 1956). In the ovaries of such females, however, many egg-chambers degenerate and fail to produce fully mature oocytes. The appearances to be described here have been seen in such degenerating egg-chambers: the conditions in the more normal eggs which may be fertilized and begin development will be described in a later article.

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MATERIALS AND METHODS

The ovaries were dissected out of females (at age 2 days) in *Drosophila* saline. Fixation was by 1 per cent. osmium tetroxide, dissolved either in buffered saline, or better, in buffered sucrose. Embedding in methacrylate was carried out in the normal way, and sectioning was done with a thermal advance microtome. The sections, mounted on Formvar grids, were examined with an electron microscope (Siemens Elmiskop I).

*The degeneration of the follicle cells*

Each egg-chamber of a *Drosophila* ovary is clothed with a follicular epithelium, the cells of which are at first all of a roughly cuboidal shape. When the oocyte begins to enlarge, at about stage 7, the follicle cells lying against it become taller and more cylindrical. From approximately the same stage onwards the vitelline membrane begins to form between them and the oocyte; as was shown in a previous paper (Okada & Waddington, 1959) the formation of the first 'vitelline bodies' is accompanied by the appearance of droplets or vacuoles in the oocyte plasm, and the material for the vitelline membrane is probably produced by the oocyte rather than by the follicle cells. However, the formation of the vitelline membrane is closely followed in time (or accompanied by) the development of the chorion, and this is certainly produced by the follicle cells, being separated from the oocyte by the already-present vitelline membrane. After the completion of the chorion, the follicle cells above it shrink and eventually disappear.

At an early stage in the formation of the vitelline membrane, the cytoplasm of the follicle cells has a relatively homogeneous structure apart from the occurrence of mitochondria. There are few indications of an ergastoplasm composed of double membranes (Plate 1, fig. 1). As the formation of the chorion gets under way, double membrane structures become more obvious. This is quite in accordance with what one observes in other differentiating *Drosophila* cells, in which ergastoplasm makes an appearance just when the cell begins to lay down its characteristic product (Waddington & Perry, 1960). The point that is particularly interesting here is that the double-membranes go on becoming more and more obvious and massive even when the cells are rapidly shrinking, immediately prior to their final disappearance; that is to say, at a time when it seems likely that the secretion of the chorion has been completed. A photo of a follicle cell at an early stage in this process is given in fig. 2 of Plate 1; the specimen has probably been somewhat over-extracted during the histological preparation, but this serves only to show up more clearly the strongly developed ergastoplasmic system. An extremely striking system of concentric double-membranes from a similar or even older cell was illustrated by Okada & Waddington (1959, fig. 11).
Changes in unfertilized eggs

The ooplasm of the mature eggs is a comparatively well-organized structure, with an outer layer of finely granular cytoplasm containing many mitochondria, and with many yolk particles, lipid particles, and granular particles of lesser or greater electron density in the more central regions. A full description has been given by Okada & Waddington (1959). In eggs which are not fertilized, one finds that after a few hours there has been a considerable rearrangement of these various constituents, of such a kind that similar particles tend to come together. The electron-light granular particles, which have been referred to as beta particles, are probably more liquid than the rest of the egg. They run together into large masses around which the mitochondria become grouped (Plate 2, fig. 4). The granular ground substance of the cytoplasm also tends to become separated into two fractions, one of a denser and one of a lighter consistency. In a late stage of degeneration, these darker areas may sometimes show an arrangement of the particles into a periodic system of parallel membranes (Plate 2, fig. 5).

Degeneration in deep-orange

In deep-orange egg-chambers the anatomical appearances of degeneration are very variable. In some cases even the polarity of the egg-chambers is reversed, so that the oocyte lies at the wrong end of it. Degeneration may begin at various stages in oocyte development, either before or after yolk formation.

In oocytes which have reached an advanced level of differentiation before degeneration overtakes them, the appearances may be rather similar to those seen in unfertilized normal eggs, in that there is a considerable segregation of the ground substance into areas of different electron density (Plate 4, fig. 11). There is also a marked development of double laminar structures which often form sets of concentric rings. In other cases the cytoplasm becomes extremely vacuolated. Some of these vacuoles probably represent badly fixed lipid particles, but it seems unlikely that all of them can be accounted for in this way, and some of them are lined by well-defined membranous structures (cf. Plate 4, fig. 10). There are also patches of a peculiar ‘fuzzy’ appearance, round which there are often one or more double profiles (Plate 4, fig. 12). These could be considered as a new type of particle, never found in healthy cytoplasm. They probably represent, however, the degenerative derivatives of one of the types of granule characteristic of the oocyte, possibly the beta granules.

The ‘annulated laminar stacks’, described as normal constituents of the egg cytoplasm, can also be seen undergoing degeneration. This involves the splitting of each lamina into two membranes, between which vacuolar swellings appear (Plate 1, fig. 3). It was pointed out in the previous paper that these laminae normally have a structure which closely resembles that of the nuclear envelope, in which the presence of annular pores is obvious but the existence
of two membranes is not very evident. The phenomena seen during degeneration make it plausible to suppose that the laminae are in fact always composed of two closely-adherent membranes even when this is not obvious.

In the deep-orange nurse cells affected by premature degeneration, the cytoplasm usually presents rather a homogeneous appearance. Some membrane-lined vesicles were found (Plate 4, fig. 10), and so were persistent mitochondria; the distribution of the latter is, however, rather peculiar even in chambers in which a fertilizable egg is being developed, and they will be considered more fully in a later paper.

The most striking feature in these nurse cells is the appearance of large masses of contorted porous membranes, which must represent the persistent remains of the nuclear envelopes. In healthy nurse cells at their fullest extent, these envelopes are very large and complexly folded (Plate 3, fig. 6). With progressive degeneration in deep-orange ovaries, the more electron-dense material inside the nucleus (nucleolar material) runs together into large more or less compact masses, while the nuclear envelope becomes fragmented (Plate 3, figs. 7, 8). The pieces of broken-up envelope rather frequently lie nearly parallel to each other, so that some regions of the complex look rather like the annulated laminar stacks (Plate 4, fig. 10). However, the whole mass of membrane is much larger and less well arranged than those stacks (Plate 3, fig. 9).

**DISCUSSION**

One point of interest in the degenerative phenomena described here is the appearance of quite elaborate structural organization in cytoplasm which is near the point of death. The segregation of components in the unfertilized eggs is, perhaps, not very surprising, but at least it indicates that inhomogeneities are not always evidence for the occurrence of vital metabolic processes. The lamellar structures found in these degenerating eggs are also a warning that caution is necessary in interpreting the functional significance of ergastoplasm-like formations, and this is reinforced by the persistence and even strengthening of typical double-membrane systems in the cytoplasm of degenerating follicle cells. It has been found that in the developing eyes of *Drosophila*, ergastoplasm usually becomes well developed in cells of any given type just at the time when the characteristic cell-product (such as rhabdomere, cornea, pigment, &c.) is being rapidly produced; and in most of these types of cells the double-membranes later regress to some extent at least (Waddington & Perry, 1960). The phenomena described in the follicle cells show that this later regression is not an inevitable accompaniment of cessation of differentiation. It appears, however, as yet too early to attempt a full understanding of the relation between the appearance of ergastoplasm and the anabolic and katabolic processes of the cell; much more information, on a wider range of differen-
Degenerative phenomena in *Drosophila* ovaries

Differentiating cells, as well as of adult cells engaged in secretion, will be necessary before we have a satisfactory basis for this. In the meantime it seems advisable to keep such appearances in mind when interpreting, for instance, the very remarkable structures described by Bellairs (1958) in yolk granules in the cells of chick embryos.

The complexes of fragmented nuclear envelopes seen in *deep-orange* ovaries are, in the first place, an indication that this structure has considerable solidity and resistance to autolysis. Perhaps the most interesting point about them is the frequent arrangement of the fragments into groups of parallel double-laminae. This could be most easily interpreted by attributing it to some relatively long-range forces, perhaps of the nature of van der Waals forces. It is noteworthy that the associations which arise in this way always involve intervals between contiguous structures which are considerably greater than the gap between the two members of the nuclear envelope, or that between the two membranes composing each lamina in the annulated laminar stacks. It seems probable that the forces holding the membranes together in paired structures, such as nuclear envelopes and the laminae in the stacks, are rather markedly different from those by which the parallel associations of fragments of nuclear envelope are brought about. One is reminded of the difference between the pairing of half-chromatids in mitotic prophase and metaphase, and the looser associations which sometimes occur in somatic pairing. The distinction between these two types of association is, however, probably not complete, since in degenerating laminar stacks the two members of a lamina may become more widely separated by the appearance of vacuoles between them, as in fig. 3 of Plate 1. The membranes composing the double structures of the ergastoplasm may also exhibit all degrees of separation, from an association almost as close as that seen in the nuclear envelope to the appearance of an extensive sac-like lumen between them. It is, however, surely significant that single and unpaired solid structures are so rarely seen in living systems. Most membranes turn out to be double-membranes, and when there are a number of pieces of such membrane, these are often found to be arranged roughly parallel to one another. The forces which bring about this arrangement must almost certainly be related to those involved in chromosome pairing and in the formation of the structures seen in unfertilized eggs.

**SUMMARY**

A description is given of degenerative phenomena in the *Drosophila* ovary. Three types of degeneration are dealt with: (1) degeneration of the follicle cells; (2) degeneration occurring in the unfertilized egg, a major feature of which is the segregation of the different constituents of the egg into comparatively large masses; and (3) degenerative phenomena in the nurse cells and oocytes of abnormal eggs produced in ovaries homozygous for *deep-orange*. 
In all these instances it is noteworthy that a considerable degree of structural organization (formation of double membranes, or sets of parallel or concentric membranes, &c.) occurs in cytoplasm which is clearly degenerating. It is suggested that such organization does not always represent the formation of functioning organelles, but may be a mere consequence of the inherent physico-chemical properties of the materials involved.

In degenerating deep-orange ovaries masses of fragmented porous membranes are found. These seem to be derived from the nuclear membranes of the nurse cells, which appear to persist even when the rest of the cell is rather completely destroyed. Fragments of nuclear envelopes tend to lie close together and parallel to one another, and it is suggested that this indicates that there is some force of attraction between them, which may be comparable to the forces involved in the formation of stacks of annulate lamellae, or of concentric or parallel double-membrane structures in ergastoplasm, and in chromosome pairing in healthy functional cells.

**RÉSUMÉ**

*Sur quelques phénomènes de dégénérescence dans les ovaires de* Drosophila

Une description est donnée des phénomènes de dégénérescence dans l’ovaire de *Drosophila*. On distingue trois types de dégénérescence: (1) la dégénérescence des cellules folliculaires, (2) la dégénérescence qui se produit dans l’œuf non fécondé dont un caractère principal est la ségrégation des différents constituants de l’œuf en masses relativement importantes, et (3) des phénomènes de dégénérescence dans les cellules nourricières et les ovocytes d’œufs anormaux formés dans les ovaires homozygotes pour le facteur orange sombre.

Dans tous les cas, il est remarquable de voir d’importants phénomènes d’organisation structurale (formation de doubles membranes, ou de séries de membranes parallèles ou concentriques, etc.) dans un cytoplasme qui est nettement en voie de dégénérescence. Il est suggéré qu’une telle organisation ne représente pas toujours la formation d’organites fonctionnels, mais qu’elle peut être une simple conséquence des propriétés physico-chimiques inhérentes au matériel considéré.

Dans les ovaires orange sombre en dégénérescence, on trouve des amas de membranes poreuses fragmentées. Ils semblent dériver des membranes nucléaires des cellules nourricières, qui paraissent persister même quand le reste de la cellule est presque complètement détruit. Des fragments de membranes nucléaires tendent à se rassembler parallèlement les uns aux autres, et l’on suppose que cela indique que des forces d’attraction les rapprochent.

Ces forces peuvent être comparées à celles qui interviennent dans la formation de piles de lamelles annulaires, ou de doubles membranes concentriques ou parallèles dans l’ergastoplasm, et aussi dans l’appariement des chromosomes des cellules saines et fonctionnelles.
REFERENCES


EXPLANATION OF PLATES

PLATE 1

Fig. 1. Parts of two follicle cells, stage 8. The external membrane is visible at the top, and from it an intercellular boundary runs down to just below the middle, on the left. At the bottom is a small area of oocyte cytoplasm, with the vitelline membrane just beginning to appear between it and the follicle cells. The follicle cell nucleus, with nucleolus, is seen at the mid-region of the right-hand cell. The cytoplasm contains many mitochondria (less dense than the cytoplasm ground substance, which is here unduly granular), but few double-membranes, though some are visible at the top of the cell and just below the nucleus. ×24,000.

Fig. 2. Follicle cell cytoplasm, stage 12, towards the end of the formation of the chorion, which is seen at the bottom right. The section does not contain a follicle cell nucleus. Ergastoplasmic double lamellae very strongly developed. ×8,000 approx.

Fig. 3. Degenerating annulated laminar stack, in cytoplasm of a deep-orange egg. ×43,000.

PLATE 2

Fig. 4. Unfertilized egg, 1½ hours after laying. The cytoplasm has segregated into large areas of different density, the light areas probably being formed from beta granules. Mitochondria (MIT) have accumulated along the margins between areas of different density, and lipid granules (represented by spaces) have also come together in groups. ×15,000.

Fig. 5. Unfertilized egg, 1½ hours after laying. The cytoplasm has again segregated into areas of different density. A light area lies above, a dark one below, with mitochondria accumulated along the boundary. The cytoplasm of the dark area has formed a series of roughly parallel bands. ×24,000.

PLATE 3

Fig. 6. Part (about one-sixth or less) of the nucleus of a wild-type nurse cell, stage 11, to illustrate the great extent of the highly folded nuclear envelope. The internal contents of the nucleus contains much scattered dark 'nucleolar' material; the cytoplasm, generally paler in tone, carries a few mitochondria and lipid granules. ×10,000.

Fig. 7. Degenerating deep-orange nurse cells, to show partial break-up of the nuclear envelope. The darker patches are presumably 'nucleolar' material. ×3,000.

Fig. 8. Persisting nuclear envelope in a deep-orange egg-chamber. Note the parallelism of several fragments, e.g. at the right near the top. ×15,000.

Fig. 9. Extensively fragmented nuclear envelope material in a deep-orange nurse cell. Contrast with the background has already been partly lost. Note the many parallel arrangements. ×36,000.
PLATE 4

FIG. 10. Structures in a degenerating deep-orange nurse cell. The annulated membranes above (NNM) are presumably derived from the nuclear membrane. Below are vesicles (VES) with associated concentric membranes, and some well-preserved mitochondria (MIT). × 43,000.

FIG. 11. A degenerating deep-orange ovarian egg. The vitelline membrane (VM) is well formed, and the egg must have been fully mature before beginning to degenerate. The contents have segregated, as in unfertilized normal eggs, and a number of concentric double-membrane rings have appeared. × 18,000.

FIG. 12. A 'fluffy body' with associated double membrane structure, in the cytoplasm of a degenerating deep-orange egg. × 33,000.

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Plate 3