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Ultrastructural indications for autosynthetic proteinaceous yolk formation in amphibian oocytes

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Summary. The formation of proteinaceous yolk is a main feature during amphibian oogenesis. The main bulk is built up by a process called heterosynthesis. The precursor complex vitellogenin is synthesized in the liver, transported by the bloodstream to the ovary, where the oocytes sequester the material by means of endocytosis. This pathway has been described in detail by many authors. The ultrastructural study of amphibian oocytes indicates on the other hand a small but distinct contribution of the oocyte itself towards yolk formation. This process has been called autosynthesis and starts before the onset of heterosynthetic activities. The cell organelles possibly involved in yolk-recursor and yolk-platelet formation are the nuclear envelope, annulate lamellae, endoplasmic reticulum, Golgi complex, GERL and mitochondria. The aim of this paper is to discuss the data, mainly of ultrastructural nature, so far accumulated during the study of autosynthesis. It is hoped to stimulate more biochemically orientated research in this field.

The formation of proteinaceous yolk in amphibian oocytes has been studied only in a comparatively small number of species. There is as yet no generally accepted view regarding the mechanisms of vitellogenesis. Early theories assumed that most of the yolk was processed within the forming oocyte; a process known as autosynthesis. More recent morphological, and especially biochemical data suggest that yolk precursors derive from an extra-oocytic source and are synthesized in the liver under the influence of estrogen (Follett and Redshaw 1974, Gerhardt 1981). The precursor complex called vitellogenin is carried in the bloodstream to the ovary where it is sequestered by the oocyte by means of endocytosis. Pinocytotic vesicles are formed there which subsequently fuse and form yolk platelets. The exact mechanism is still not fully understood. This pathway, known as heterosynthesis, is well documented by many authors, and provides the main bulk of material for yolk formation (for literature see Follett and Redshaw 1974, Bergink and Wallace 1974, Brummett and Dumont 1976, Wiley and Dumont 1978, Knowland 1978, Wallace 1978). Many of these authors are inclined to think that the uptake of yolk precursors, produced by cells other than the differentiating oocyte, is the only mechanism of yolk formation. This theory has often been overemphasized (Wallace et al. 1972, Wallace 1978, Korfsmeier 1978). Some authors, however (Yew 1969, Spornitz and Kress 1971, 1973, Kress and Spornitz 1972, Wartenberg 1973, Kress 1978, Ward 1978a, b)), have always maintained that there is more than one possible route of yolk-platelet formation and that different cell organelles may be involved in the synthesis of amphibian yolk, as is the case in many invertebrates, mainly molluscs, polychaetes and crustaceans (Ganion and Kessel, 1972, 1980, Bottke 1973, Terekado 1974, de Jong-Brink et al. 1976, Hill and Bowen 1976, Lui and Connor 1977, Eckelbarger 1979, Bilinski 1979, Schade and Shivers 1980, Zerbib 1980). This combined production by extra- and intraoocytic structures has been called auto-hetero-synthesis.

The contribution of cell organelles should not be underestimated. New support for this latter view in amphibians was recently provided by Kessel and Ganion (1980a, b) describing the contribution of cell organelles towards yolk formation in *Necturus maculosus* and *Rana pipiens* and by results obtained by the author for species not hitherto investigated, namely *Rana erythrea, R. graeca, R. occipitalis, Bombina bombina, Bufo bufo, Ptychadaena mascariensis, Hyla arborea, Pleurodeles waltl, Triturus cristatus, T. marmoratus, and Salamandra salamandra terrestris.*

It is our intention to review the results so far published concerning autosynthesis of yolk in amphibians, in the light of new knowledge about the organelles involved.

Each ripe yolk-platelet consists of a crystalline center, of a phosvitin-lipovitellin complex, an electron-dense granular matrix and a surrounding membrane (fig. 1). Small amounts of carbohydrates and enzymes are also to be found, especially in the matrix (Slaughter and Triplett 1976, Wallace 1978, Wittenberg et al. 1978, Robertson 1979) as well as some DNA (Kirsch-Volders 1974). The latter seems to be taken up by adventitious engulfment together with the vitellogenin but plays no role in embryonic development (Opresko et al. 1979).

Yolk platelets synthesized and formed mainly by oocyte organelles in an early stage of development are called *primary yolk-precursors* or *yolk-precursors I.* Yolk platelets, formed mainly by large quantities of yolk-proteins sequestered by pinocytosis are called *secondary yolk-precursors* or *yolk-precursors II.* In the ripe oocyte, however, we find only one population of yolk platelets, so far as morphology and centrifugation experiments are concerned (Greenhouse and Morrissey 1974).

Organelles which we have to consider and discuss in this paper as possibly taking part in the primary yolk-precursor (precursor I) and actual yolk-platelet formation are:

1. The nuclear envelope
2 Annulate lamellae
3. Endoplasmic reticulum
4. Golgi complex and GERL
5. Mitochondria

This discussion will be followed by descriptions of

6. Multivesicular bodies I
7. Yolk-precursors I
8. Inclusion bodies within mitochondria and endoplas mic reticulum as found in the oocytes of some amphibian species.

In very young oocytes the organelles are sometimes condensed and located in a paranuclear position. This arrangement is referred to as Balbiani body or yolk-nucleus. It does not exist in all species and where it occurs its compositions varies considerably with respect to the organelles included. There exists no direct connection with yolk formation. As already proposed by Anderson (1974), the term is confusing and will be abandoned in this paper.

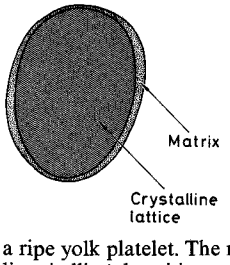


Figure 1. Structure of a ripe yolk platelet. The main part consists of a crystalline lattice of lipovitellin/phosvitin and is surrounded by a dense granular matrix and a membrane.

1. Nuclear envelope (NE)

The NE, more or less smooth in the very young oocyte, becomes extensively folded during the growth of the cell; such folding increases the surface area of the organelle. The outer (sometimes even the inner) membrane (Kessel 1965) appears to be engaged in considerable activity during the course of oogenesis. This activity, as seen in all species investigated, is reflected in blebbing (fig. 2). A large amount of blebbing of the outer membrane takes place during early stages of development and seems to contribute towards different cell organelles (Kessel 1968a, Franke 1974a, Harris 1978, Zbarsky 1978). The outer membrane may have some areas with ribosomes attached to its outer surface, while other areas are smooth. This means the NE may be composed of functionally different membrane areas and therefore constitutes a mosaic of variable components like the granular and the smooth endoplasmic reticulum (ER). The vesicles blebbing off in oocytes or in other cells show no visible content of any sort, but since ribosomes are attached to part of the NE, it is possible that they may be involved in the synthesis of intracisternal secretion just as in granular ER. Kessel and Decker (1971, 1972) found nucleoside diphosphatase as well as thyamine pyrophosphatase activities in the NE of *R. pipiens* oocytes. Both enzymes are markers for synthetic activities, the latter being involved in phospholipid metabolism. Taking all these facts into consideration, the latter is especially interesting in view of the lipoprotein content of the yolk platelet. The NE's ability to contribute towards other cell organelles such as ER (Kessel 1969a), annulate lamellae (Kessel 1968a, Wischnitzer 1974), Golgi apparatus (Weston et al. 1972) and multivesicular bodies (Kilarski and Jasinski 1970) has been described. Therefore, it does not seem far-fetched to suggest that some of the NE vesicles may be directly incorporated into forming yolk-precursors I, where either their content or the lipoproteins of the NE membrane itself are of importance. This situation, with early precursor stages lying very close to the NE, and seeming to incorporate blebbing vesicles can be found in most of the amphibian oocytes we investigated. It is demonstrated especially clearly in *T. cristatus* (fig. 3).

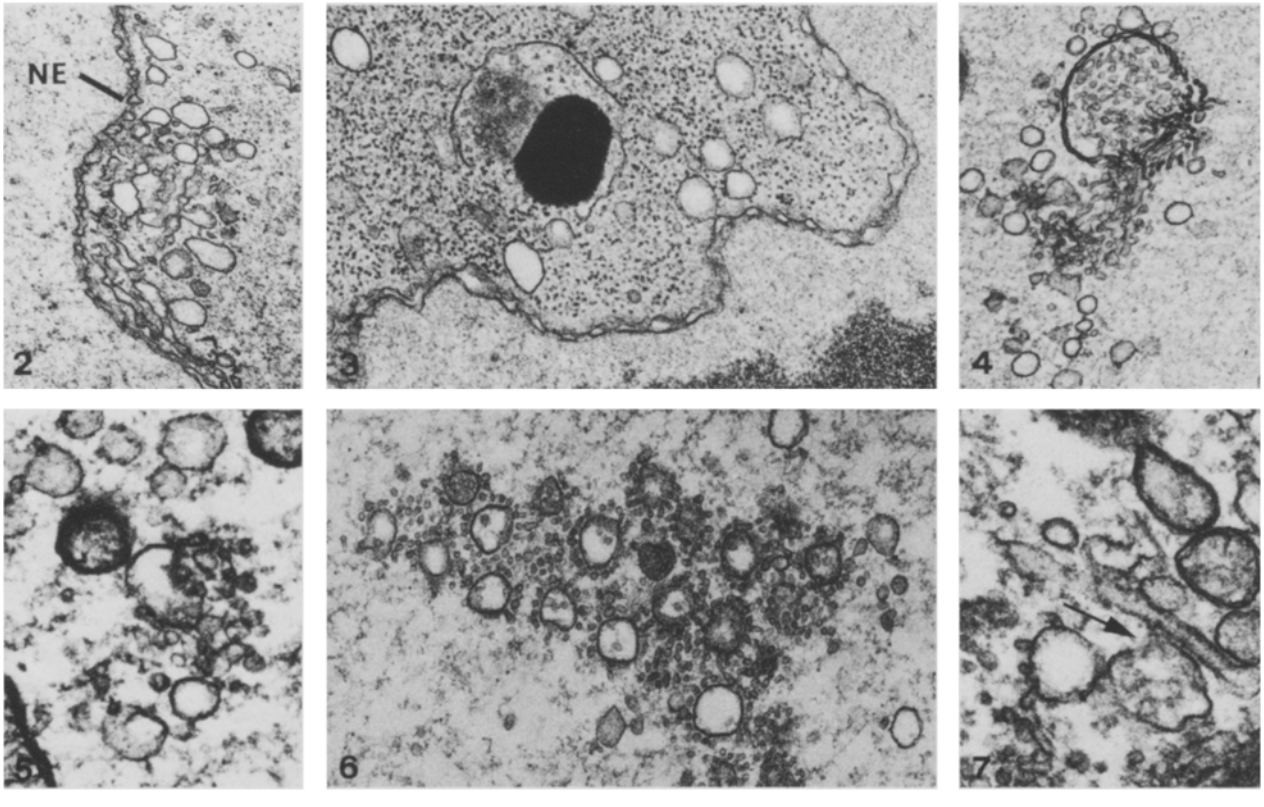
2. Annulate lamellae (AL)

These structures are widespread in germ cells, oocytes and spermatids (Sun et al. 1977) or other fast growing cells such as tumor cells (Sun and White 1979).

The AL derive from the nuclear envelope. In younger oocytes a large amount of blebbing at the NE indicates that this membrane must be in an extremely dynamic state of membrane synthesis and membrane flow (fig. 2). The outer membrane forms rows of single vesicles which move further into the ooplasm and

make up lamellae forming pores or annuli often surrounded by denser material. The lamellae are arranged in stacked parallel arrays (figs 10, 11). Both the length of the individual lamellae as well as the number of lamellae in a single stack are extremely variable. The AL already appear in the previtellogenic oocyte and the number and the size of stacks increase with the size of the oocyte and are more usually located in the animal pole. However, there is no definite correlation between the stage of the oocyte and AL formation (Kessel 1968a). The morphological features are not always the same, as already shown by the important study of Kessel (1968a). These differences in morphological features may depend to some extent on the fixation, as shown by Kessel (1969a). This can be confirmed by our studies; using glutaraldehyde and OsO_4 results in more laminar structures of different length, while with OsO_4 alone more individual vesicles result. On the other hand, distinct differences of the AL structure among the species are noticeable (figs 10, 11, 13, 14). The ends of the individual lamellae often expand and vesiculate as shown by Kessel (1968a), by Kress and Spornitz (1972) and in figures 10 and 11. These vesicles look exactly like isolated ER vesicles. The AL can be continuous either with granular or smooth ER or have ribosomes attached to them. Obviously the AL possess certain structural characteristics of both the NE and ER. The arrangement and features of the AL may vary:

- the AL can exhibit the typical structures with pores or annuli, as found in most of the oocytes (fig. 10);
- the lamellae and pores can be linked with dense granular or fibrillar material, as is the case in *Bufo* (fig. 10) and shown by Kessel (1968a);
- the lamellae may be invested with ribosomes, as seen in *Bombina* and described for *Xenopus* (Steinert et al. 1974);
- the pores may sometimes be missing, the lamellae interrupted without typical annuli. This type can be seen in *Bombina* (figs 13, 14) and has been described in *Xenopus* (Steinert et al. 1974) and in echinoderm oocytes (Kessel 1968a) as well as for spermatids (Sun et al. 1977) and tumor cells (Sun and White 1979);
- the AL can often be seen in continuity with ER lamellae, forming loops and ring-shaped systems. Some zones show an intermediate character between AL and ER (*Bombina,* fig. 13);
- single AL lamellae may form circles with just one or only a few annuli; a situation which we found in



Figures 2–7. Fig. 2. Nuclear envelope (NE) exhibiting extensive blebbing *(R. occipitalis).* × 19,500. Fig. 3. Vesicles stemming from the outer nuclear membrane. Some of them are taken up by a yolk-precursor I *(T. cristatus).* × 34,500. Fig. 4. Cluster of vesicles, some of them fusing to form a surrounding membrane thus producing a multivesicular body (mvb) *(T. cristatus).* × 27,300. Fig. 5. Single ER-vesicles incorporating small vesicles to form mvb *(Ambystoma mexicanum).* × 43,000. Fig. 6. Field with numerous stages of mvbs *(Salamandra salamandra).* × 30,000. Fig. 7. ER-cisternae with budding ER-vesicle (arrow), taking up material from a nearby dictyosome *(Ambystoma mexicanum).* × 43,000.

Ambystoma (figs 8, 9) and which was found in *Ciona* oocytes by Kessel (1968a).

The function of AL is still debated. Up to the present it could not be isolated and its enzymes are not yet known. The AL is thought to be a first-line reservoir for membranes needed in the fast-growing oocyte. Another question which needs to be pursued is this; does the AL contain nuclear-derived products and information in order to control remoter regions of the oocyte? Is it a possible route of transport between nucleus and cytoplasm? Anderson (1974) states that the AL does not take part in yolk formation, but this may merely be a problem of nomenclature. The problem is whether the vesicles taken up by yolk-precursors I in the immediate vicinity of AL lamellae should still be considered as part of AL or already as ER vesicles (fig. 10). In *Ambystoma* single AL circles may contain vesicles or condensation products in the interior just as in precursors (figs 8, 9). In *Bombina* some AL stacks are located directly beneath the oolemma (fig. 14) and seem to contribute towards the increasing oocyte surface just as is described for Golgi vesicles in *Triturus* (Giorgi et al. 1976). This participation of AL has been demonstrated in *Discoglossus*, a species belonging to the same family as *Bombina* (Denis-Donini and Campanella 1977). It helps to form the dimple, an area specialized for the fertilization process, which needs a local increase of about 11% in membrane surface.

3. Endoplasmic reticulum (ER)

The ER is represented in the developing oocyte by numerous smooth and rough surfaced vesicles or short lamellae scattered throughout the cytoplasm. Whether there is a more vesicular or a more lamellar type of ER depends again upon the method of fixation (Kessel 1971, Kress and Spornitz 1972), an observation which holds true for the newly-investigated species as well. The vesicular type of ER is derived mainly from the blebbing of the outer nuclear membrane. In *T. vulgaris* we find dense granular material surrounded by ER vesicles adjacent to the forming face of the Golgi apparatus. Out of this granular material seems to result a de novo formation of membranes for an increasing number of ER vesicles, vesicles which probably contribute towards the forming-face of the Golgi (Spornitz and Kress 1973). In *Ambystoma* we find vesicles of ER budding from tubular ER. These vesicles incorporate smaller vesicles from the surroundings and grow to multivesicular bodies (mvbs) (fig. 7). In other cases single big ER vesicles take up adjacent smaller vesicles (figs 5, 6) leading to mvb-formation too. Yet another type of ER behavior is demonstrated in figure 4. From an aggregation of small vesicles some of the outer ones start to fuse and form a membrane around the centrally placed ones. The resulting picture looks very similar to ER loops which form an autophagic vacuole by encircling the organelles to be destroyed (Trump 1975, Novikoff 1976, Novikoff and Novikoff 1979).

The ER can, as already mentioned, be continuous with the AL and the GERL (see below).

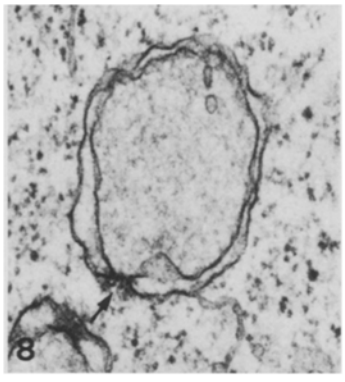
As in the NE, Kessel and Decker (1971, 1972) found nucleoside disphosphatase and thyamine pyrophosphatase within the compartments of the ER. The ER, sphatase the smooth variation, is known to be involved in the synthesis of non-protein components such as cholesterol and steroid hormones, and in other steps of lipid synthesis. Its contribution towards yolk-precursors I which are building up lipoprotein-complexes would therefore not be surprising. This contribution of ER vesicles towards mvbs and yolk-precursors I has been described for *Bufo valliceps* (Yew 1969), for *R. esculenta* and *R. temporaria* (Kress and Spornitz 1972), for several species mentioned by Wartenberg in his review (1973), for *Ambystoma* (Kress 1978) and for *Necturus maculosus* and *R. pipiens* (Kessel and Ganion 1980a, b). The metabolic ability of the ER to break down organelles and compounds in autophagic vacuoles may be active in mvbs and precursors as well. Vesicles which have been taken up totally into ER vesicles and mvbs soon become unravelled and are turned into dense granular material (compare section mvbs and precursors).

4. Golgi-Apparatus and GERL

What seemed to be clearly understood, the structure of the Golgi-apparatus and its contribution towards storage and packing of secretory products and lysosome formation, is now revealed to be much more complicated and involved.

The available biochemical evidence confirms the existence of chemical and enzymatic heterogeneity between the various Golgi elements. In the 'trans'-face of the Golgi the enzymes nucleoside-diphosphatase (NDPase) and thyamine-pyrophosphatase (TPPase)

Figures 8–14. Fig. 8. Circular-shaped annulate lamellae with one pore (arrow) enclosing dense cytoplasm and small vesicles *(Ambystoma mexicanum).* × 50,000. Fig. 9. Circular-shaped annulate lamellae enclosing dense granular material *(Ambystoma mexicanum).* × 72,000. Fig. 10. Stack of annulate lamellae which give off vesicles at their ends. Some of these vesicles are integrated into yolk-precursors I; others contribute towards the ER *(T. cristatus).* × 19,500. Fig. 11. Large stack of annulate lamellae with regularly spaced pores and dense material between the arrays of lamellae. Continuity into ER cisternae and into GERL-area (*) can be seen *(Bufo bufo).* × 18,000. Fig. 12. Concentrically arranged cisternae of rough endoplasmic reticulum, as found in great numbers in *Bombina bombina.* × 26,000. Fig. 13. Annulate lamellae in concentric layers and precursor-lamellae of different length continuous with the ER (arrow) *(Bombina bombina).* × 17,000. Fig 14. Annulate lamellae situated directly underneath the oolemma. Vesicles seem to move towards the oocyte surface which shows numerous microvilli *(Bombina bombina).* × 24,500.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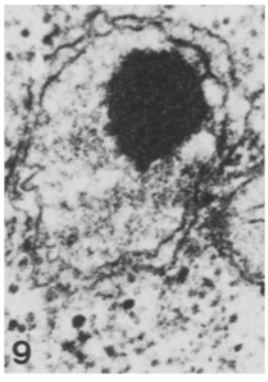
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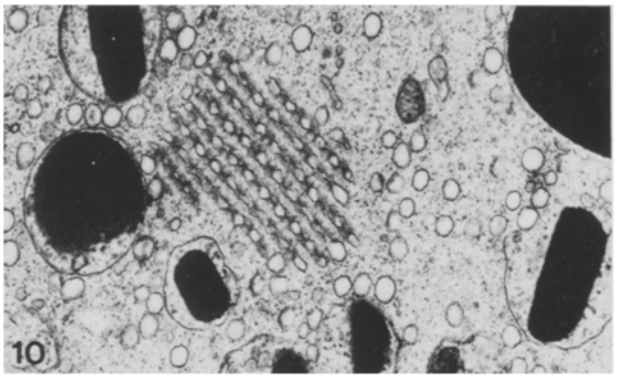
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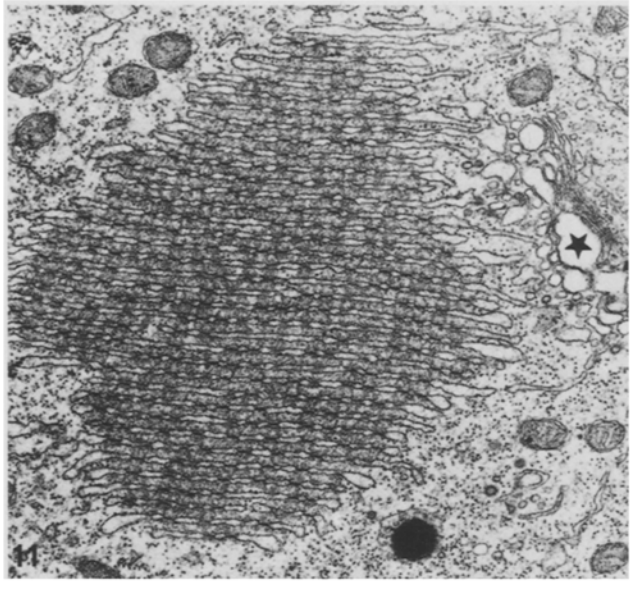
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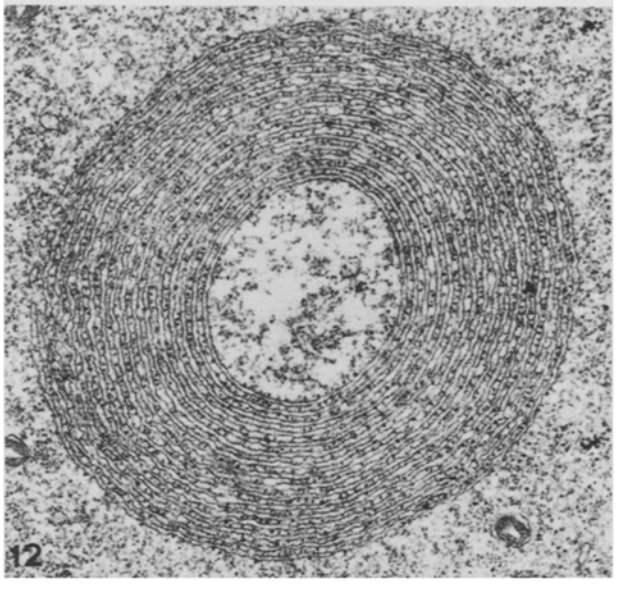
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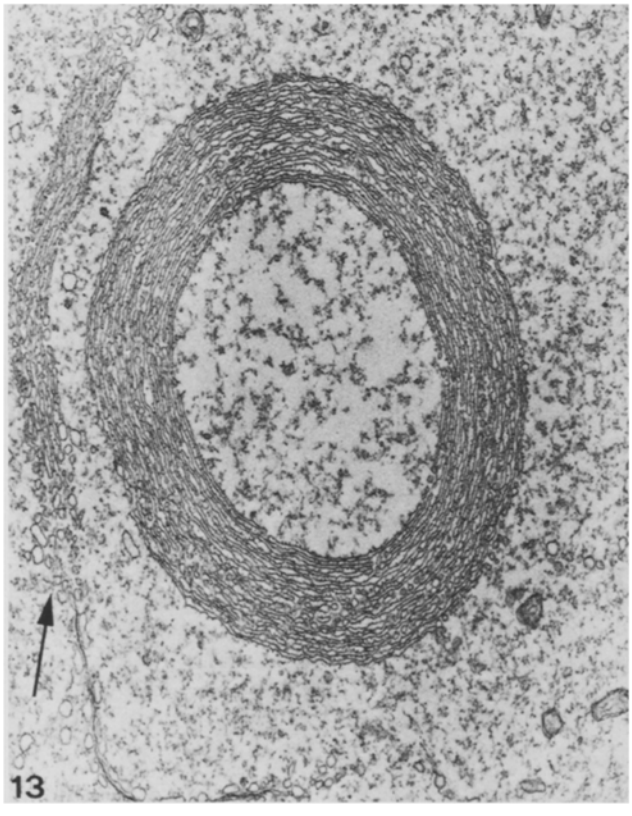
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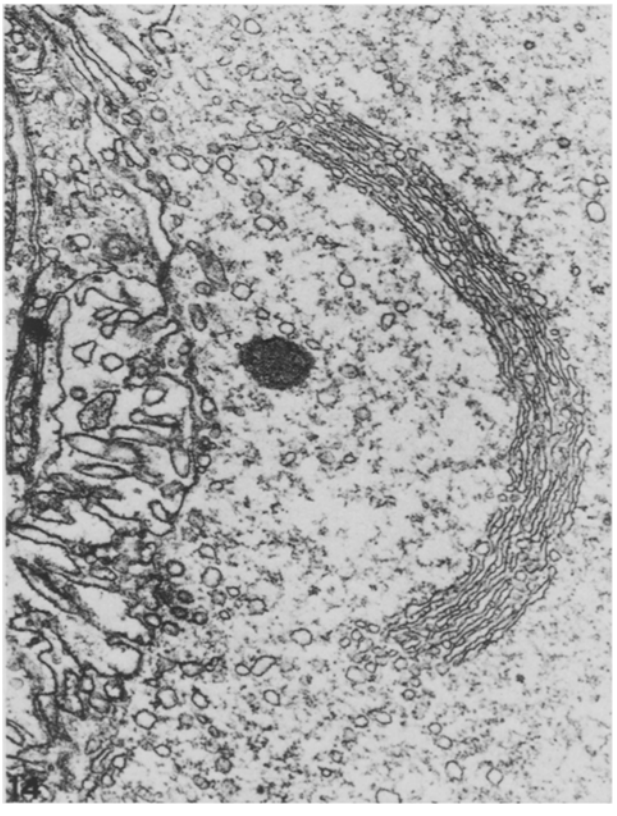
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are typical, while in the innermost saccules, in the condensing as well as in the coated vesicles of the inner Golgi area, acid-phosphatase is the marker enzyme (Friend 1969, Kessel and Decker 1971, 1972, Novikoff 1976). Novikoff (1964) named this acid-phosphatase active region with the acronym GERL. This name indicates its localization in the Golgi area, its origin from the ER and its participation in lysosome formation. According to Hand (1980) the GERL is 'lying adjacent to the trans Golgi saccules or may be separated by a space containing vesicles or other structures'. The membranes of GERL seem in general thicker than those of Golgi- or other ER saccules and are often coated. The GERL may show continuity with various forms of vesicles, secretory granules or other parts of the ER. Golgi saccules have a more irregular cisternal width and are closely associated with one another within a stack. The functional continuity between Golgi and ER seems not always to be direct, but rather to be mediated through small transfer vesicles.

GERL shows a system of anastomosing tubules around the periphery of the cisternal parts of the Golgi. It would appear as though the so-called inner region of the Golgi area in routinely prepared specimens is in fact a section through GERL, this means ER; the size and extent of which has obviously been underestimated (Hand 1980). In secretory cells in which it has been identified, GERL seems to play an important role in packing proteins into secretory granules. If this is so, the Golgi is obviously bypassed, a situation which led Novikoff (1976) to question the function of the Golgi itself.

These new findings on the Golgi structures have to be taken into consideration when reading the descriptions of processes attributed to the Golgi in the past. Without the necessary cytochemical tests it is impossible to differentiate clearely between Golgi and GERL and their possible contribution not only towards yolk precursors but also towards cortical granules and especially to premelanosome formation (Mishima et al. 1979). Do Golgi elements contribute towards GERL? It is difficult to distinguish the origin of pinched-off vesicles even after cytochemical analysis. Variation in the maturation stage of the oocyte, of the organelle concerned or its stage of activation may produce varying results (Kessel and Decker 1972).

In the past only a few authors denied a connection between the Golgi apparatus an yolk formation (Hope et al. 1964, Ward 1968a). Most authors thought it possible or even proved a participation of the Golgi (in the old sense of the term), because Golgi complexes are numerous and very active in the growing oocyte and pinched-off vesicles can be found in great numbers in the adjacent area and in precursors (Massover 1971a, b, Kress and Spornitz 1972, Spornitz and Kress 1973, Wartenberg 1973, Anderson 1974, Giorgi et al. 1976, Kress 1978, Kessel and Ganion 1980a, b).

Looking retrospectively in the literature at the pictures of Golgi complexes and the results obtained by cytochemistry in the light of what we know now, some of the illustrations and explanations fit the idea of GERL better than that of the Golgi structure (Eppig and Dumont 1972, 1974, Kessel and Decker 1971, 1972, Kessel and Ganion 1980a, b).

To demonstrate the origin of vesicles within yolk precursors the OsO_4-zinc-iodide stain technique has been used (Giorgi et al. 1976, Kress, unpublished). Giorgi et al. have already discussed the theory that even vesicles without any common origin with the Golgi could be stained with this method; however, in the end, they favour the idea of the Golgi-derived nature of these vesicles. Some of his pictures, however, and my experiments with *Pleurodeles* oocytes, give the impression that not so much Golgi but rather GERL material is stained (figs 16, 17). The vesicles are transported to yolk-precursors I (fig. 17) although yolk-precursor II, formed in the pinocytotic area under the oolemma, may show stained inclusions as well (fig. 18).

In many species Golgi structures in the trans area, as well as GERL exhibit an arrangement of saccules and vesicles with thicker membranes, these membranes may be coated and sometimes be surrounded by granular material. A direct continuity with the ER can often be discovered (Spornitz and Kress 1973, Kress 1978).

The biochemical activities attributed to the Golgi apparatus, such as polysaccharide synthesis, glycoprotein formation, packing of lipoproteins and enzymes, would make it still plausible for Golgi vesicles to be integrated into yolk precursors I, because the compounds mentioned can be found in varying amounts in the ripe yolk platelets (Slaughter and Triplett 1976, Wallace 1978, Wittenberg et al. 1978, Robertson 1979).

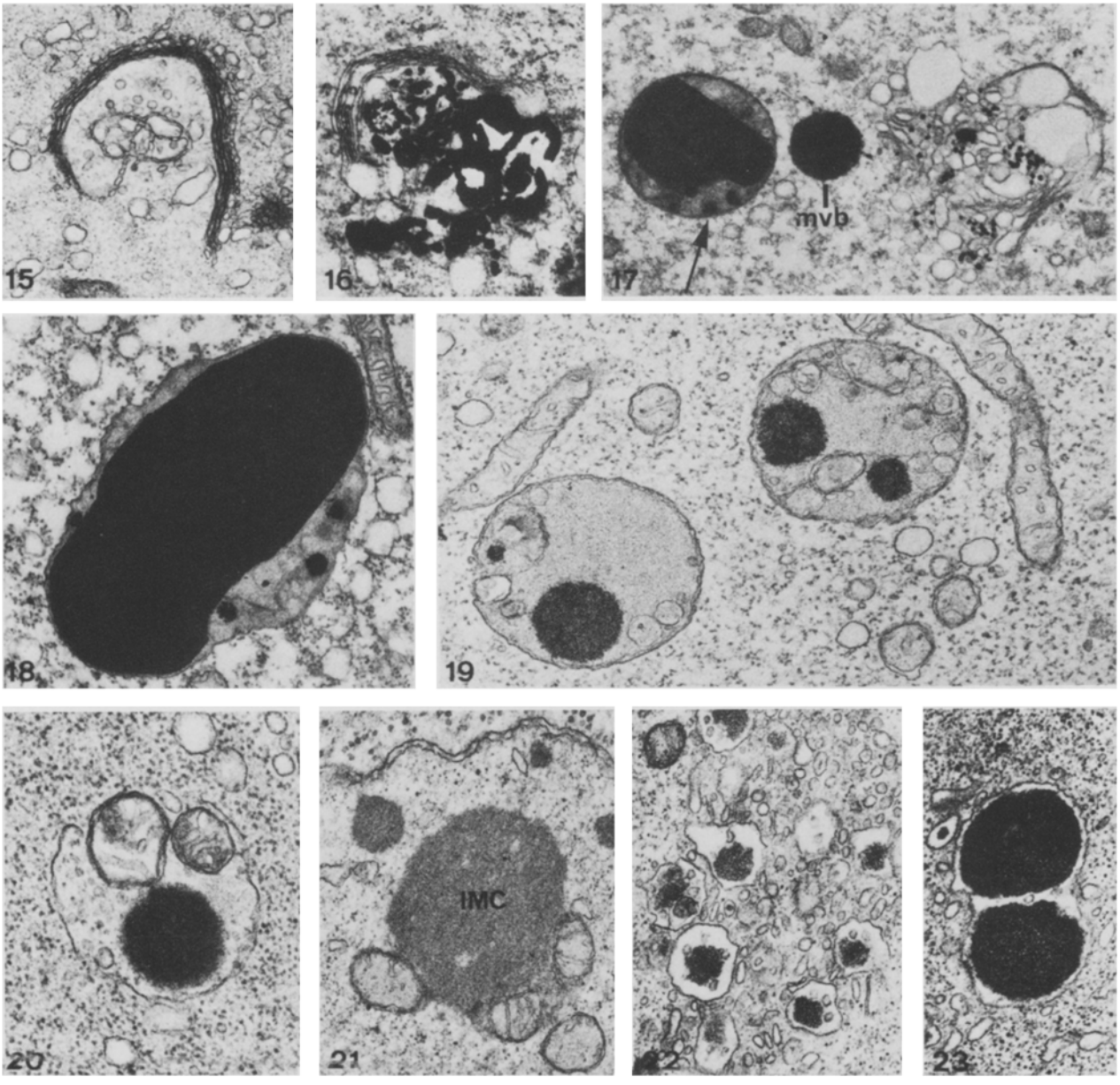
Morré and Vigil (1979) demonstrate in rat hepatocytes a dynamic model of the Golgi apparatus in which membranes differentiate progressively across the stack of cisternae. Friend (1969) detects Golgi as well as ER vesicles in forming mvbs in rat epididymidis. These results all show that the discussion of what activities are performed by the Golgi apparatus and which by the GERL is still an open question. This holds true to an even greater extent when we have to decide to which organelles the vesicles contributing towards mvbs I and precursors I belong. More detailed investigations in this direction will be necessary, e.g. with the antibody-enzyme method, which could help to detect vitellogenin production within cell organelles of the oocyte (Thompson et al. 1980).

Giorgi et al. (1976) observed that Golgi material seems to be incorporated into the surface membrane,

the oolemma. This observation can be confirmed, especially for *Ambystoma* (Kress 1978).

5. Mitochondria

A major portion of the mitochondrial population of a young oocyte is found circumferentially around the nucleus, usually arranged in clusters of sometimes enormous dimensions (Billett and Adam 1976). Near the nucleus they are often associated with electron-dense material called 'intermitochondrial cement (IMC) or nucleolus-like bodies (Kessel 1969b). The product, stemming from an extrusion of the nucleus, can often be seen in connection with the pores and seems to contain RNA (Webb 1976). Its function is still enigmatic. As the oocytes grow and differentiate, the mitochondrial mass increases 50–100-fold (Callen et al. 1980), and the mitochondria become more randomly distributed. The IMC disappears.



Figures 15–23. Fig. 15. Golgi-apparatus with GERL area *(Pleurodeles waltl).* × 26,000. Fig. 16. Golgi-apparatus and GERL area stained with OSO_4-zinc-iodide method *(Pleurodeles waltl).* × 33,000. Fig. 17. GERL vesicles stained with OsO_4-zinc-iodide have been forming mvbs (one pictured here) and have been taken up into a yolk-precursor I (arrow) *(Pleurodeles waltl).* × 17,000. Fig. 18. Precursor II, placed in the outer zone of the cortex, with OsO_4-zinc-iodide stained vesicles in its interior *(Pleurodeles waltl).* × 17,000. Fig. 19. Growing mvbs I acquire electron-dense matrix areas and turn into precursors I. The incomplete dissolution of incorporated vesicles may lead to a great number of different membrane configurations *(Pleurodeles waltl).* × 30,000. Fig. 20. Uptake of 2 mitochondria into a precursor I *(T. cristatus).* × 39,000. Fig. 21. Mitochondria arranged in a cluster around electron-dense intermitochondrial cement (IMC) close to the nuclear envelope *(Ambystoma mexicanum).* × 27,000. Fig. 22. Mvbs II, which are formed by fusing pinocytotic vesicles, are located close to the oolemma *(Ambystoma mexicanum).* × 28,500. Fig. 23. Precursor II formation, resulting from growing mvbs II *(Ambystoma mexicanum).* × 27,000.

The extent to which mitochondria take part in yolk-precursor I formation is still an open question. A transformation into yolk-precursors of parts of some mitochondria has been described in the case of *Xenopus* (Balinsky and Devis 1963, Spornitz and Kress 1971) as well as for *Bufo valliceps* (Yew 1969) and *R. pipiens* (Ward 1978).

The uptake of whole mitochondria, or parts of them, into yolk-precursors has been mentioned by Kessel and Ganion (1980a) and has been observed in *T. cristatus* (fig. 20). This situation is found only very occasionally and it seems to happen more or less by accident during the uptake of other vesicles into the growing precursor.

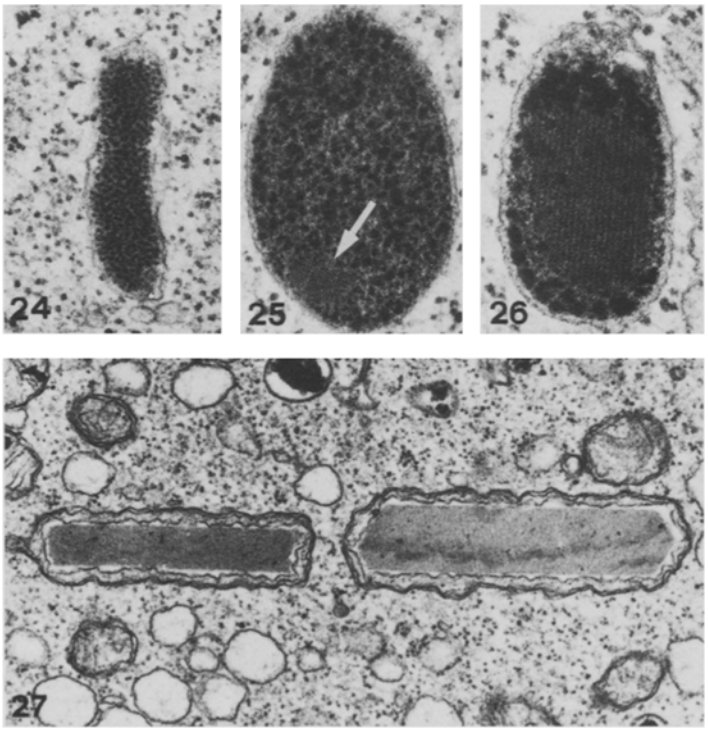
6. *Multivesicular bodies (mvb)*

This term is commonly used to describe a vesicle of varying size containing small vesicles. These organelles may be numerous in many cells, especially in differentiating amphibian oocytes.

In the amphibian oocyte we have to distinguish 2 categories of mvbs according to the 2 pathways of yolk-platelet formation. Early in oogenesis (at the stage called previtellogenic), before the oolemma exhibits an overall set of microvilli, mvbs form mostly in groups near the NE more or less centrally located within the oocyte. These mvbs I grow into yolk-precursors I (figs 6, 19). At a later stage when the oolemma possesses an extensive arrangement of microvilli, the pinocytotic activities increase drastically (vitellogenic stage) and the pinocytotic vesicles also fuse to form mvbs (mvbs II) (figs 22, 23). They grow later into yolk-precursors II.

The origin and fate of mvbs I have often been called elusive (Ward 1978a). According to the descriptions of the cell organelles in the oocyte, as given in the previous sections of this paper, and the discussion about their possible contribution toward yolk-precursors, mvbs seem to be very complex structures. Growing evidence has emerged in the literature that they are formed to a great extent by the Golgi apparatus (using the old term) (Kress and Spornitz 1972, Spornitz and Kress 1973, Giorgi et al. 1976, Kress 1978, Kessel and Ganion 1980a, b). The new observations that part of the former Golgi complex belongs in reality to the ER (GERL), indicates that the ER seems to provide the main bulk of vesicles for building up the mvbs. This contribution of the ER is surely not uniform. There must be differences in the composition of the vesicles depending on their origin either from the NE or AL or from the GERL area. The extent to which real Golgi vesicles of NE or AL vesicles are taking part in mvb-formation has to be investigated in more detail.

Wartenberg (1973) called the mvbs 'complex cytosomes', a very accurate term because of the multitude of cell organelles involved, but his term is not identical with our mvbs I because he did not distinguish between the mvbs of the 2 different pathways (mvbs I and mvbs II).



Figures 24–27. Fig. 24. Dilatated ER-cisternae with distinct granular content *(Ambystoma mexicanum)*. × 54,000. Fig. 25. Areas of crystallization appear in a subsequent stage (arrow) *(Ambystoma mexicanum)*. × 63,000. Fig. 26. ER inclusion body with crystalline lattice and granular matrix *(Ambystoma mexicanum)*. × 84,000. Fig. 27. Intramitochondrial inclusion bodies within cristae *(R. erythrea)*. × 28,600.

7. Yolk-precursors I

The original individual vesicles in the mvbs disappear, and the center becomes increasingly electron dense. The changing mvbs, now called yolk-precursors I, incorporate vesicles into their growing membranes; however, they often exhibit an incomplete membrane covering obviously due to the fact that many vesicles from the surroudings are taken up wholly (Massover 1971a, b, Kress and Spornitz 1972, Spornitz and Kress 1973, Kress 1978, Kessel and Ganion 1980a, b). The membranes of the incorporated vesicles often unravel and form all sorts of whorls within the mvbs and especially within the precursors. At some stage of development, which differs according to the species, the electron dense matrix starts to form crystalline centers within the precursors. The way the lipoproteins of the incorporated membranes are used for the construction of the crystalline lattice is one of the unsolved problems. We cannot rule out some uptake of pinocytotic material into yolk-precursors I (Wartenberg 1973, Spornitz and Kress 1973). This latter cannot be excluded since the experiments of Herzog and Farquhar (1977) and Gonatas et al. (1977) showed that endocytotic vesicles were integrated into Golgi cisternae, into GERL or into lysosomes (Geisow 1980). This would implicate a pathway back into a membrane reservoir and a possible indirect way for extra-oocytic material to be inserted into yolk-precursors I. In *Ambystoma*, primary and secondary yolk-precursors exhibit the same reaction towards the application of pronase solution. As long as no crystalline areas exist, no change can be seen. As soon as the precursors show crystalline centers these disappear when treated with pronase; the same observation has been made on ripe yolk-platelets (Kress 1978).

Inclusion bodies

In the family Ranidae and some closely related species *(Rhacophorus)* we find crystals within quite a number of mitochondria. They lie mainly within the cristae or sometimes in the matrix of the mitochondria and they have for a long time been considered to be yolk. They have been described as yolk-precursors I or II (Lanzavecchia 1960, Massover 1971a, b, Kessel 1971, Kress and Spornitz 1972). However, the inclusions exhibit distinct differences from real yolk platelets:

- they have a crystalline lattice which is similar, but the center-to-center spacing of the lattice is not identical with that displayed by yolk platelet crystals in the same oocyte (Spornitz 1972, Kress and Spornitz 1974);
- the phosphorus content and the reaction towards pronase are different (Spornitz 1972);
- the inclusions have no granular matrix around the crystal.

For these reasons it is better to avoid the term yolk (Anderson 1974, Kress and Spornitz 1974), and use the neutral term inclusion bodies. We would not consider them as part of the autosynthetic pathway towards yolk formation as described above. Their function remains unclear.

How do they form? The idea that they could be coded by the mDNA and mRNA cannot hold true because:

- the inclusions are found more often within the cristae than in the mitochondrial matrix, the site of mDNA and mRNA;
- the size and codon capacities of the 2 m-nucleic acids are, as we know today, not sufficient to control the information for such macromolecules.

The inclusion bodies are mostly formed before the pinocytotic activities in the oocyte start. No author has, to our knowledge, ever described the uptake of vesicles into mitochondria. On the other hand, the connection between the ER and the mitochondrial exterior membrane has been described by Kessel (1971), and observed, especially in *Ambystoma*. But this phenomenon is found too infrequently to give a

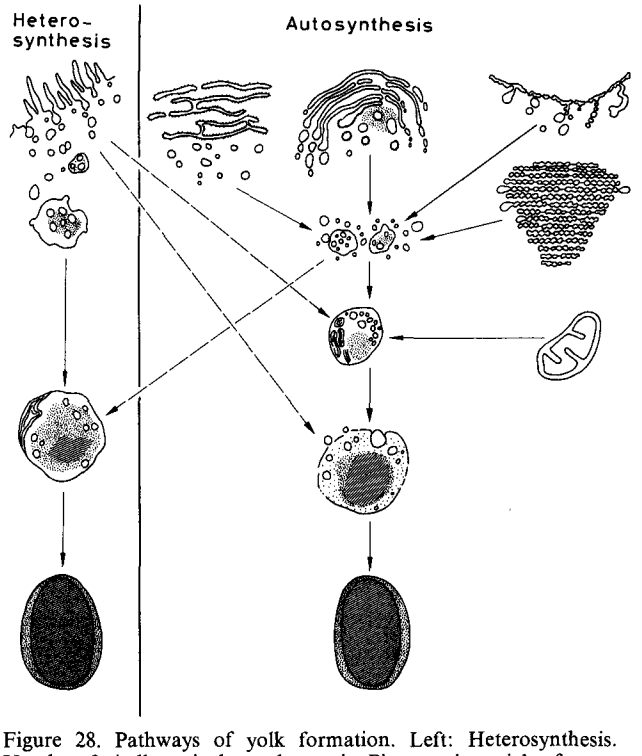


Figure 28. Pathways of yolk formation. Left: Heterosynthesis. Uptake of vitellogenin by endocytosis. Pinocytotic vesicles fuse to form mvbs II and grow into precursors II with an increasing crystalline zone. The final results are ripe yolk-platelets. This pathway contributes the main bulk of yolk material. Right: Autosynthesis. The diagram summarizes the results of this paper. The possible involvement of cell organelles such as Endoplasmic reticulum, Golgi complex including GERL, nuclear envelope, annulate lamellae and mitochondria towards yolk formation via mvbs I and precursors I is depicted. Their quantitative contribution towards total yolk production is not yet known.
The 2 pathways are not strictly separated (interrupted arrows). Pinocytotic vesicles seem to be included into forming precursors I, as well as vesicles of the autosynthetic pathway into precursors II.

satisfactory explanation of the way these inclusions are formed.

Not only mitochondria are the site of inclusion bodies; they can be found in the ER too, as is the case in *Ambystoma* (Kress 1978). The granular material does not always crystallize and there is always some granular matrix left around the crystal. But while pronase attacks the crystalline lattice of the real yolk platelet, the lattice structure remains intact after pronase treatment of the ER-inclusion – a similar effect to that seen in mitochondria. In some crustaceans, part of the yolk formation is in fact the work of the ER, as described by Kessel (1968a), Hinsch and Cone (1969), Ganion and Kessel (1972), Bilinski (1979). In *Ambystoma* the situation could be similar.

Concluding remarks

Yolk formation in amphibians seems to be a more complex process than has previously been thought (fig. 28). Several different mechanisms operate in order to accumulate the nutritive material required. Even if the material for yolk production must ultimately be derived from outside the oocyte, 2 pathways can be distinguished:

a) uptake of whole yolk proteins from the circulatory system by pinocytotic vesicles (heterosynthesis);

b) uptake (diffusion or active transport) of low molecular weight yolk precursor molecules into the oocyte to be changed into primary yolk precursor by the combined effort of numerous cell organelles (autosynthesis).

This theory of an auto-heterosynthetic process still leaves unsolved the problem of the precise extent to which the 2 systems are involved. In invertebrates such as polychaetes (Eckelbarger 1979), crustaceans (Ganion and Kessel 1972, 1980, Lui and Connor 1977, Bilinski 1979, Schade and Shivers 1980, Kessel and Beams 1980, Zerbib 1980) and molluscs (Bottke 1973, Terekado 1974, de Jong-Brink et al. 1976, Hill and Bowen 1976) the autosynthetic pathway of yolk formation is a common one, either by itself or together with heterosynthesis. Because of the great amount of yolk which has to be formed in amphibians (the amount is even greater in many fishes and in reptiles and birds, where no signs of intra-oocytic participation have been reported so far), the processing of yolk products, once performed by cell organelles alone, may perhaps have been delegated and heterosynthesis has become the main pathway. Yet the ability of the organelles to form yolk has not been lost; part of the contribution still stems from cell organelles, as described here. Since eventually only one population of yolk platelets exists in the ripe oocyte, the pathways of yolk formation are probably not strictly separated. There seems to be some uptake of Golgi- or ER-vesicles as well as small mvbs into yolk-precursors II (figs 18, 28) as well as the insertion of pinocytotic material into yolk-precursors I (Wartenberg 1973, Spornitz and Kress 1973) (fig. 28). The concept that membranes are integrated into the cell surface via exocytosis, either discharging material or carrying receptors, and are later recycled via endocytosis, which internalizes attached material back into internal membrane systems, has been reviewed by Morré (1981). This new membrane shuttle concept has to be seriously considered and tested as a possible transport route involved in the yolk forming process.

Nothing has been mentioned here about other possible storage materials such as fat or glycogen often included within the term yolk. Comparing the different species which we investigated, there seems to be a varying amount of proteinaceous yolk, fat or glycogen. It may have to do with different living conditions. Fat and glycogen will be the subject of another paper.

Attention has to be turned in future to experiments which will clarify the actual part played by the different cell organelles in yolk synthesis. The approach to amphibian oogenesis from a more comparative point of view could provide more insight into the different possibilities of yolk formation.

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SPECIALIA

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A novel xanthone as secondary metabolite from *Centaurium cachanlahuen*

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Summary. From the plant *Centaurium cachanlahuen*, swertiaperenine **1**, swercherine **2**, decusatine **4**, oleanolic acid **5** and the xanthone 1,8-dihydroxy-2,3,4,6-tetramethoxy-9H-xanthone **3** were isolated and characterized.

From our studies on *Centaurium cachanlahuen* (Mol) Robinson, a Chilean native species[2], we report the isolation of 3 known xanthones and a novel one, and also of the triterpen oleanolic acid.

Dry tissues of the plant were extracted with cold methanol. The extract was concentrated under vacuum, then diluted with water, and was finally successively extracted with petroleum ether (60-80 °C), toluene, dichloromethane and ethylacetate.

Chromatography of the fraction soluble in dichloromethane on a column of silica gel 60 (Merck, 0.063-0.2 mesh) using petroleum ether as eluant and step-wise elution with increasing proportions of ethyl acetate afforded the following compounds: Swertiaperenine[3] (**1**), swercherine[4,5] (**2**) the xanthone **3**, decusatine[6] (**4**) and oleanolic acid[7] (**5**) (see fig. 1).

Elution with petroleum ether/ethylacetate = 95/5 gave a yellow crystalline compound of melting point 168-169 °C.