one[7] (V) was prepared from 16α-methyl Reichstein's Substance S[8,9] by reduction[10] of the 21-tosylate of the latter. Compound V was also prepared in a seven-stage synthesis from 16α-methylpregnenolone acetate[2] *via* the intermediate 5α,6α-epoxy-17α-hydroxy-16α-methylpregnanolone benzoate [m.p. 228–230°, $[\alpha]_D = -49°$ ($CHCl_3$)]. Acetylation of the 17α-hydroxyl group provided the 17α-acetate (VI). Bromination[11] of the enol ether of the latter with subsequent dehydrobromination with calcium carbonate in dimethylformamide[11] gave 16α-methyl-6-dehydro-17α-acetoxyprogesterone (VII). Dehydrogenation of VI and VII with 2,3-dichloro-5,6-dicyanobenzoquinone[12] gave 16α-methyl-1-dehydro-17α-acetoxyprogesterone (VIII)[1] and 16α-methyl-1,6-*bis*dehydro-17α-acetoxyprogesterone (IX), respectively.

Whereas a large increase in antiinflammatory activity has been associated with the introduction of a C16-methyl group in the corticoid series[13], in no instance have we observed a pronounced increase in oral or subcutaneous progestational activity of the methylated progesterone over the corresponding desmethyl compound.

*Zusammenfassung.* Mehrere Derivate des 16α-Methylprogesteron wurden synthetisiert und biologisch geprüft. Die Einführung einer C16α-Methylgruppe ergab keine gesteigerte Progesteronaktivität in den getesteten Verbindungen.

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## Structural Basis of Nucleolar Function

Due to the lack of a definite concept as to whether structure or function is basic to the study of biological organization of cells[1,2], an integrated approach is the accepted operational choice of biologists to-day. Accordingly, studies on structure and function are expected to proceed concurrently in a balanced sequence. Recently, some valuable contributions have been made on the (hetero)chromatin- nucleolus- protein synthesis relationships[3–7]. Curiously, however, the exquisite details of the fine structure of nucleolus are still lacking.

In the present study[8], some structural facets have been described by making a survey of extensive cytological changes that take place in the ophidian oocytes during their formation and development. The ophidian ovary was selected as being cytologically the least explored ovary. Moreover, the structural differentiation of the oocyte is perhaps the simplest. Yet, the biocytological processes cover a wide range of activities illustrative of cellular growth[9,10]. The study has revealed some architectural patterns peculiar to nucleolar function.

*Nucleolar behaviour.* A recounting of the chain of events during the formation and maturation of the oocytes would reveal some interesting features of nucleolar organization. The early oocytes may be distinguished by the possession of a prominent coiled spireme within the nucleus during the prophase stages. At these stages, there is no indication of the presence of nucleolus. Later, a single nucleolus makes its appearance enmeshed in a loose reticulum of achromatic strands in close association with the suspended chromatin material (Figure 1). Thus, in the early stages of its formation, the nucleolus appears homogeneous. It is negative to the Feulgen reaction and has, therefore, no indications of DNA constituents. In advanced oocytes, commensurate with the growth of the oocyte, the nucleolus grows to a considerable size and soon begins to bud off several smaller bodies. The budding process of the nucleolus is shown in Figures 2 and 3. This process of actual budding has rarely been described. The nucleolar derivatives are more or less of the same size. But exceptions to this may be found. The daughter nucleolar bodies come to lie near the nuclear membrane and are eventually extruded into the cytoplasm. In Figure 3, the much enlarged nucleolus is shown to be differentially stained. The central part is deeply stained while the outer part is lighter. This difference can be interpreted as due to variable concentrations of its constituent substances *or* to its duplex nature. Figures 2 and 3 also show in a clear manner the formation of daughter nucleolar bodies from the principal nucleolus, indicating that these daughter nucleolar bodies are not formed from any other source. The nucleolar organizers, which in earlier phases might be importantly concerned in the origination of nucleolus, do not seem visibly to participate in the formation of these nucleolar derivatives. There is no increase in the size of the nucleolar bodies after they reach the cytoplasm, neither do they multiply. They appear to be completely dissolved in the ooplasm without having directly transformed into any kind of yolk material. The extruded nucleolar bodies provide the cytological mechanism for the exchange of certain chemical substances from the nucleus to the cytoplasm (Figure 4). Presumably, these activating chemical

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substances are essential for the elaboration of yolk material and in protein synthesis.

*Functional significance.* The main questions in connection with nucleolar function are: (1) the influence and role of 'nucleolar organizer'; (2) the function of nucleolus as a store-house or as an active seat of RNA synthesis; (3) the cytochemical heterogeneity of the nucleolus; and (4) the mode and significance of nucleo-cytoplasmic exchanges of nucleolar products. The structural evidences provided here are somewhat helpful in elucidation of some of these problems.

(1) *Nucleolar organizer.* The absence of nucleolus in early oocytes may, perhaps, provide an incipient evidence that in initial stages at least the origination and function of nucleolus is influenced by the closely associated chromatin. In later phases, however, independent centres of protein and RNA synthesis are established.

(2) *Active role.* The nucleolus undergoes active changes in form, size, and staining capacities. The actual process of nucleolar budding is shown in Figures 2 and 3, thereby indicating that the nucleolus is not merely a store-house of RNA but acts as an active seat of RNA and protein synthesis. The secondary nucleoli are extruded into the egg cytoplasm and provide the agency by which RNA from the nucleus is laid down into the cytoplasm. In the slug oocyte[11] also the secondary nucleoli represent independent centres of protein and RNA synthesis produced during the course of oocyte growth.

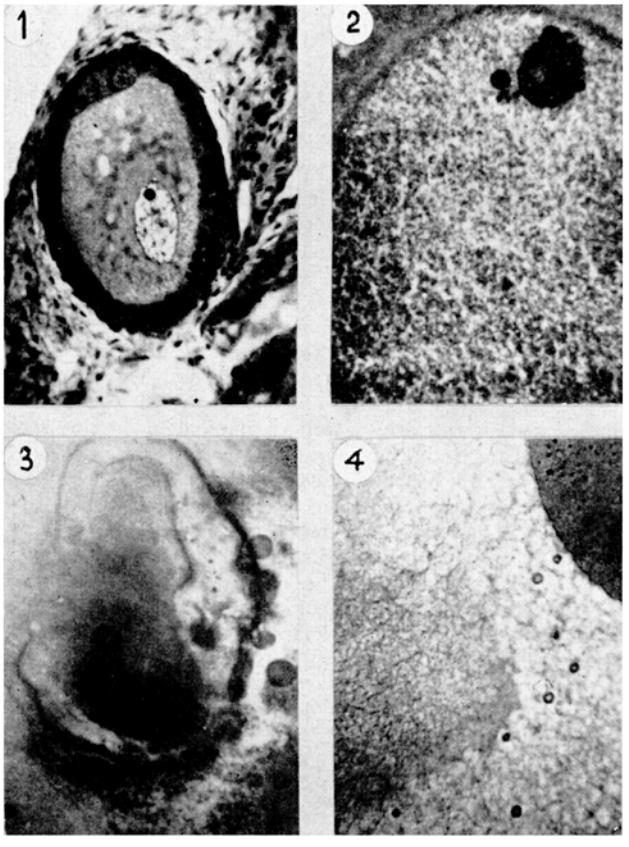


Fig. 1. Cocyte showing early nucleolus inside the nucleus (*Thamnophis ordinatus ordinatus*). Bouin, Mann's methyl blue eosin. × 140. Fig. 2. An advanced oocyte showing budding of the nucleolus inside the nucleus to form daughter nucleolar bodies (*Thamnophis ordinatus ordinatus*). Bouin, Harris alum hematoxylin. × 280. Fig. 3. The duplex principal nucleolus in the process of budding inside the nucleus of an advanced oocyte. Several nucleolar bodies are formed in the process (*Thamnophis ordinatus ordinatus*). Bouin, methylene blue eosin. × 630. Fig. 4. An advanced oocyte showing extrusion of the secondary nucleolar bodies from the nucleus into the cytoplasm (*Natrix piscator piscator*). Bouin, Mann's methyl blue eosin. × 140.

(3) *Heterogeneity.* The duplex structure of the principal nucleolus is shown in Figure 3. The heterogeneity may be due to the non-homogeneous distribution of RNA. The less intensely stained outer portion indicating a lower concentration of RNA. Another possibility could be the differences in the mechanism by which the dye attaches to two possible types of RNA. LOGAN and DAVIDSON[12] have designated a nuclear $RNA_1$ and $RNA_2$ based on differential solubility.

Recently, the growth of the slug oocyte has been studied cytochemically[11] with respect to DNA, RNA, histones, basic proteins, mucopolysaccharides, and phospholipids. It has been found that the two classes of nucleoli found in the oocyte nuclei are of different chemical composition.

(4) *Nucleo-cytoplasmic interchanges.* It seems that the extrusion of nucleolar bodies rich in RNA provides a means for the transfer of the nucleic acids from the nucleus into the cytoplasm for protein synthesis. If this be true, the extrusion of the daughter nucleoli is a very significant process because, through these nucleolar bodies, protein or protein precursors are discharged into the cytoplasm. The present work provides direct structural evidence to show that there is an exchange of materials between the nucleus and the cytoplasm, but the nature and role of the extruded materials need to be further investigated. The passage of RNA from the nucleus to the cytoplasm in amoeba[13] has also been reported. It is reasonably certain that the microsomal RNA-protein particles are major sites of protein synthesis[6]. But there is as yet no explanation of how this function is geared to nuclear and cytoplasmic physiology.

The highly specific effects exerted by the nucleus on the cytoplasm are generally thought to be mediated by means of the RNA. There is clearly a requirement for RNA in the cytoplasmic synthesis of protein. RNA is synthesized most actively in the nucleolus, and can pass to the cytoplasm. However, it remains uncertain whether some RNA is synthesized in the cytoplasm also. Recent experiments with nuclear transplantation in amoeba[14] suggest that specific protein synthesis occurs in the nucleus. These interesting results demonstrate that some protein of the nucleus is transferred to the cytoplasm and then back to the nucleus. Earlier studies on enucleated cells have shown that protein synthesis is reduced in the absence of the nucleus, indicating that at least some protein synthesizing ability is immediately dependent upon the presence of the nucleus. The consensus of recent literature[15] is that both the nucleus and cytoplasm carry on protein synthesis.

*Zusammenfassung.* Es scheint ein fundamentaler Zusammenhang zwischen Struktur und Funktion des Nukleolus vorhanden zu sein. Die Spätphase des Hauptnukleolus hat eine Doppelstruktur. Die beobachtete Nukleolusvermehrung zeigt, dass der Nukleolus ein aktives Gebiet für RNS und Proteinsynthese ist. Die Übertragung der Nukleolusstoffe vom Kern ins Cytoplasma wird diskutiert.

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