Neben den «coated vesicles» und auch weiter innen kommen kleinere und kleinste Bläschen ohne coat mit verschieden dichtem Inhalt vor. Diese stehen oft in enger Beziehung zu Dottervorstufen (Figur 6). Von den beschriebenen Vesikeln lassen sich die Vakuolen des ER gut unterscheiden, indem sie durch ihre Grösse, unregelmässige Formen und geringe Dichte auffallen (Figur 6).

Während der Bildung der Eihülle können in ihr verschiedene Zonen festgestellt werden. Direkt auf das Oolemma folgt eine helle, praktisch leere Stelle (Figur 7, I), darauf eine elektronendichte, grobflockige Lage (II) und aussen, unter der Basallamina, eine feinkörnige, elektronendurchlässige Schicht (III). Zwischen I und II, II und III werden Übergänge beobachtet. In allen Zonen sind noch einzelne vom Oolemma ausgehende Microvilli erhalten geblieben (Figur 7). Erst das reife Ei besitzt eine homogene Hülle ohne Microvillireste[6].

Diskussion. Die Simultanfixierung vereinigt Vorteile sowohl von O als auch von G/O[11]. Es wurde gezeigt, dass die Nachbehandlung in wässriger Uranylacetatlösung den Kontrast der Strukturen steigert[12] und teilweise auch noch als Fixationsmittel wirkt[14]. Im Falle von *O. moubata* lassen sich mit der TF die Ovozyten und die darin vorkommenden rickettsienähnlichen Mikroorganismen[14] gleichermassen gut fixieren. Membransysteme werden ausgezeichnet dargestellt und verschiedene Vesikeltypen kann man an der Peripherie der Ovozyten unterscheiden.

Gewisse Zellelemente, wie Diaphragmen der Kernporen und Microtubuli können nach TF nicht mehr gefunden werden. Im Gegensatz dazu sind Microtubuli in Nierengeweben[11] und in Leukozyten[12] nach Simultanfixation vorhanden.

Das ER ist teilweise zusammenhängend und verzweigt, hingegen besteht es nach O oder G/O aus einzelnen Vakuolen. Die Ribosomen haben polymorphe Formen mit heterogener Struktur und nicht klar definierten Umrissen (nach O oder G/O sind es scharf begrenzte, rundliche Partikel). Welche Gestalt dem lebensfrischen Zustand des ER und der Ribosomen näher kommt, bleibt noch abzuklären.

Um die Zelldynamik während der Eireifung zu untersuchen, bringt uns die TF nicht bedeutend weiter. Hier müssen gezielte Markierungen mit Tracer-Substanzen helfen[10].

Summary. The triple-fixation (simultaneous fixation in a mixture of glutaraldehyde and osmium tetroxide and post-treatment in aqueous uranylacetate) is especially suitable for the demonstration of membranes and to distinguish different types of vesicles in the oocytes of the soft tick *Ornithodorus moubata.*

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On the Lay-Out of the Midgut Rudiment in *Loligo pealei* (LeSueur)

Since a developmental study published by KORSCHELT[1] in 1892, the mid- and hind-gut complex of the cephalopoda is unanimously considered as a non-ectodermic formation, which in later embryonic stages becomes connected with the ectodermic stomodaeal complex and with the anal ectoderm.

Very much attention has been paid to the questions arising about the origin of the mid- and hind-gut rudiment: whether it is the only remainder of entoderm, or part of it, or an actual mesentoderm, arising entirely from the mesoderm. A final conclusion seems impossible for various reasons that cannot be discussed in the present note.

As to its morphology, the early mid- and hind-gut rudiment has been described – in KORSCHELT's and subsequent studies – as a small medioventral epithelial plate lying on the yolk syncytium and consisting of very few cells at the beginning; this epithelial rudiment was assumed to expand laterally and to migrate posteriorly in order to fuse at the caudal end of the internal yolk sac with the stomodaeum.

A description of the early organogenesis of the mid- and hind-gut complex of *Octopus vulgaris* recently given by the author[2] depicts a somewhat different process that is in fact common to both *Octopus* and *Loligo*[3]: the early rudiment is already a compound one in the shape of a transverse epithelial band, laterally expanded from the beginning of its morphological appearance. The similarity between the morphogenetic action of this mes(ent)odermic rudiment and the rudiment of the central nervous system, within the entire organogenesis of the embryo, is emphasized in the above-mentioned paper[2].

With ARNOLD's[4,5] absolutely convincing hypothesis of an inductive role of the egg cortex in mind, one could assume that the large size of the early rudiment is due to a corresponding lay-out of its particular organ-determining area on the cortex ('morphogenetic inductive map'[5]) rather than to an already completed growth of an originally small rudiment. The latter is in fact inconceivable for such early stages. Conclusive results about this, however, could only be expected of an experimental approach.

Some ligation experiments have therefore been made on eggs at very early embryonic stages in order to eliminate different parts of the cortex in the presumptive mid- and hind-gut area. The present results, although relying on a small number of specimens, provide us with a serious support of the ideas based on the earlier morphological observations[2].

Material and methods. Eggs of animals spawning in the laboratory were prepared for ligation by stripping off the jelly layers of the egg strings. The ligations were made with fine hair on eggs (surrounded by the chorion only) at early embryonic stages[5], between 8 and 13 (staging according to ARNOLD[6]). Parts of the cortex in the presumptive ventral or ventrolateral area thus were completely separated from the egg. After periods of 5 or 6 days, the ligated specimens were preserved in Bouin's fixative, embedded either in Epon 812 or in Paraplast and

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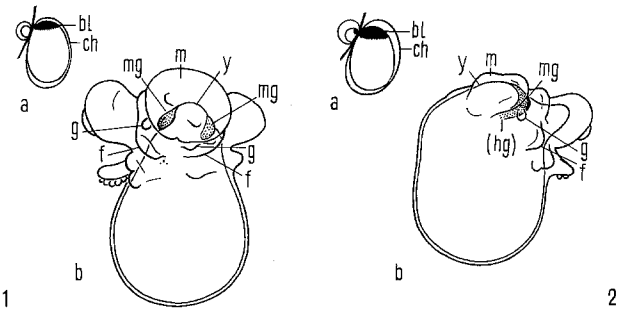

Fig. 1. Ventral view of a specimen ligated on the ventral side at stage 8–9 (a), preserved 6 days later (b). The hind-gut complex is lacking, the mid-gut parts are well developed on either side (reconstructed from cross sections).

Fig. 2. Ventral view of a specimen ligated ventrolaterally at stage 10 (a), preserved 6 days later (b). Mid- and hind-gut parts are lacking on the ligated side; the mid-gut rudiment is well developed on the other side and tapers off into the hind-gut area (reconstructed from cross sections).

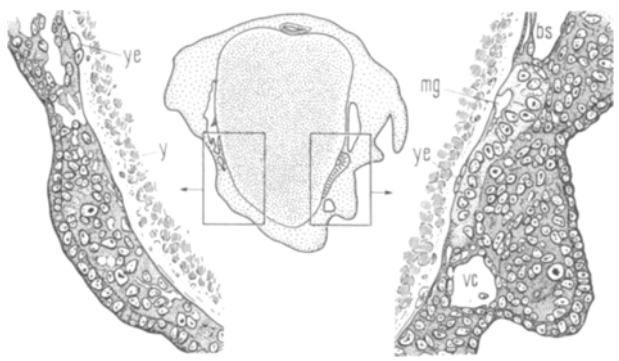

Fig. 3. Cross section of a specimen ligated ventrolaterally at stage 12–13, preserved 5 days later. On the ligated side, no trace of differentiation into an epithelium is visible; on the intact side, a distinct mid-gut epithelium is differentiated and begins to form a hepatic tube (camera lucida drawings of Epon section).
bl, blastodisc; bs, blood sinus; ch, chorion; f, funnel; g, gill; hg, hind-gut; m, mantle; mg, mid-gut; vc, vena cava limb; y, yolk; ye, yolk epithelium nuclei.

sectioned in thicknesses below 1 μ on an ultramicrotome or 6 μ on a regular microtome. The sections were stained with Azure Blue or Masson's Trichrome. The reconstructions shown in Figures 1 and 2 were made from drawings (camera lucida) of 6 μ cross-sections[7].

Results and discussion. Histological analysis of 10 specimens that had developed well after ligation showed that elimination of the ventral cortex in the presumptive hind-gut area inhibits the formation of the intestine and ink sac only while the rudiments of the actual mid-gut (stomach, caecum, hepatopancreas) form on either side (Figure 1). Removal of a lateral part of the cortex leads to the formation of an incomplete rudiment lacking the parts corresponding to the ligated area (Figures 2 and 3). In the live embryo, the presence of the respective parts of the rudiment appears in the constriction of the yolk which is absent at the site of the ligation.

If the original rudiment were a small one, restricted to a medioventral spot as KORSCHELT described it, the cortex area by which it is determined would have to be as small. Elimination of this ventral induction area would inhibit the entire mid- and hind-gut formation, whereas removal of the more lateral parts of the cortex would not primarily affect the formation of a complete mid- and hind-gut complex[8].

Zusammenfassung. Die Abschnürung von Teilen des ungefurchten Eicortex in der prospektiven Mittel- und Enddarmregion bei *Loligo pealei* führt zum Fehlen der entsprechenden Darmteile in späteren Stadien. Dies zeigt, dass die zusammenhängenden Anlagen des Mittel- und Enddarmkomplexes von Anfang an in Form eines lateral weit ausladenden Epithelstreifens vorliegen.

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A Possible Role of Microtubules in the C Cells Secretory Mechanism

It is well known that Calcitonin is released following hypercalcemia[1] and that the same condition causes a discharge of the secretory granules of the C (parafollicular) cells of the thyroid[2]. Evidence has been presented suggesting that the secretory granules are the site of Calcitonin storage[3]. In in vitro organ culture, loss of Calcitonin (demonstrable with immunofluorescence) and degranulation of the dog thyroid C cells follows an increase of the calcium level of the culture medium[4,5].

It is not yet known how Calcitonin containing granules are released. We investigated the mechanism of secretion of C cells both in normal and cultured (stimulated by high calcium concentration of the medium) dog thyroid. We paid special attention to a possible role of microtubules in the mechanism of secretion.

Thyroids were obtained from 5 dogs and either a) directly fixed in 3% buffered glutaraldehyde, post-fixed in OsO_4 1%, dehydrated and embedded in Durcupan ACM Fluka; the stain was performed with uranyl acetate during dehydration and lead citrate on the sections; or b) cultured for 36 h at 37 °C in organ culture according to the technique previously described[4,5]. The calcium level in the culture medium (TC 199 Wellcome) was increased to 7.5 or 10.2 meq/l by adding $CaCl_2$. After

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