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Synthesis of the Mesoglea by Ectoderm and Endoderm in Reassembled Hydra

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ABSTRACT The structure and synthesis of the mesoglea was investigated in "reassembled" hydra—hydra regenerating from ectoderm and endoderm previously isolated from each other and then recombined. During tissue isolation and reassembly the mesoglea remains attached to the endoderm. It is observed to be quite elastic and resilient. The mesoglea disappears by 6–8 hr after reassembly, having apparently been digested by endoderm. "New" mesoglea is undergoing synthesis by 12 hr after reassembly. Its trilaminar appearance at this time suggests an origin from both epithelia. Interepithelial contact, by cell processes of epithelial cells, is reestablished within the mesoglea between 24 and 48 hr after reassembly. Mesoglea appears normal 48 hr after reassembly. Autoradiographic experiments, performed during the reassembly manipulations, conclusively demonstrate that the mesoglea originates from both epithelia. Mesoglea precursors, amino acids, are incorporated within the mesoglea about 5–6 hr after initial acquisition by epithelia, but subsequent turnover of these amino acids is slow.

The mesoglea of hydra (Hydrozoa, Cnidaria) is a thin (0.5–2.0 μm, Haynes et al., '68), acellular layer situated between the ectoderm and endoderm of this diploblastic organism. When isolated, the mesoglea appears as a fibrous "ghost" that retains the tubelike shape of the polyp (see Campbell, '74; Epp, '79). Burnett and Hausman ('69) and Hausman and Burnett ('69, '70) described the light microscopic structure of such isolated mesoglea as a sheet of rectolinearly-arranged collagen and elastin fibers (0.3 μm diameter) embedded in an amorphous gel.

Ultrastructurally, the mesoglea appears quite different, that is, as a more or less homogeneous mass of fibrils (6–8 nm diameter) embedded within the amorphous gel (Davis and Haynes, '68; Haynes et al., '68). This appearance is similar to that of a typical vertebrate basement membrane (Day and Lenhoff, '81). Barzanski et al. ('75) analyzed hydra mesoglea biochemically and found its amino acid and neutral sugar composition to be similar to that of vertebrate basement lamina.

The function of the mesoglea in hydra is indeed that of a basement membrane in that it provides structural support for the two epithelia. It is also the site of contact for ectodermal and endodermal cell processes and thus for intercellular communication between the epithelia (Hufnagel and Kass-Simon, '76). In addition, it possibly plays other morphogenetic roles, perhaps directing cell migration (Burnett and Hausman, '69; Shostak et al., '65) or regulating form in hydra (Campbell, '79).

Little is known, however, about the origin and synthesis of the mesoglea in hydra. Only Hausman and Burnett ('72) have actually investigated this. Their method involved providing intact hydra with tritiated proline, an amino acid precursor of collagen, then subjectively estimating by autoradiography and light microscopy the timing and amount of incorporation of label in various parts of the animal. While their results differed somewhat between regenerating and intact hydra, and while epithelia were labeled much more readily than mesoglea, they believed label was detectable within both epithelia, as

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seen in cross-sections of intact hydra, about 4 hr before it was detectable within isolated mesoglea from similarly-treated, but different animals. This suggested to them that both epithelia contribute to the mesoglea. However, while both epithelia may have actively picked up proline, it is also possible that only one may have done so. This layer might then have rapidly transferred it to the other, as endoderm would do with digested food. Moreover, only one epithelium may have actually used proline for mesoglea synthesis. For instance, ectoderm might use proline for nematocyst capsule formation instead (see Lenhoff et al., '57).

This paper further investigates the structure, origin, and synthesis of the mesoglea in hydra. It describes work utilizing a technique whereby ectoderm and endoderm of hydra are separated, recombined, and a new hydra regenerated—a "reassembled" hydra. Light and electron micrographs made during such reassembly and regeneration provide many interesting observations about the dynamic morphology of the mesoglea—its degradation, synthesis, and association with epithelial cells. Further, autoradiographic experiments, performed in conjunction with reassembly and regeneration, definitively show that both epithelial layers contribute to synthesis of the mesoglea.

MATERIALS AND METHODS

Cultures of *Hydra attenuata* Pallas used in these investigations were maintained in Loomis' solution (Loomis and Lenhoff, '56) at 16°C and fed three times per week with freshly-hatched *Artemia* nauplii.

Separation and recombination of epithelia

The method used for separation and reassembly of ectoderm and endoderm of hydra (see also Smid and Tardent, '82, '84) involves removing the "head" and "foot" by cutting and then soaking the body column in a mixture of two parts cold 1% procaine hydrochloride [prepared by Limmattal Hospital Zurich: 10.0 gm procaine chlorate (Hoechst), 7.0 gm NaCl and 8.0 ml of 0.1 N HCl per liter distilled water] and one part Loomis' solution. This solution causes independent contraction of the longitudinal ectodermal and circular endodermal muscle fibers. Such contractions lead to loosening of the epithelia from each other. After about 30 min the ectoderm and endoderm are sufficiently separated to allow the endodermal tube to be carefully withdrawn from the surrounding ectoderm. In a more delicate manipulation the endoderm may then be reinserted in either its own or another, donor, ectoderm (Fig. 1a). Culture of the resulting recombinant for several days leads to the regeneration of a normal hydra (Fig. 1b,c).

Radioisotope labeling

For radioisotopic labeling of tissues, 24-hr starved hydra of adult size but without buds were injected (David, '83) twice, with a 1-hr interval, with 1–2 μl of a tritiated amino acid mixture (TRK-440, Amersham International). This mixture provides not just proline, but 15 of the 19 amino acids identified in hydra mesoglea by Barzansky et al. ('75). Two injections were performed, since there was the possibility that amino acids might be utilized quickly by epithelial cells. (This caution proved to be unnecessary as it has been found that a single such injection labels mesoglea heavily). One hr after the final injection the gastrovascular cavity was flushed with culture solution. Any reassembly manipulations were performed at this time.

Autoradiography

For autoradiography, isolated mesogleas or semi-thin sections of recombinants were individually mounted on microscope slides and covered with Kodak AR-10 stripping film. After incubation for 3–4 weeks at 4°C, these preparations were developed and observed with phase-contrast microscopy.

Isolation of mesoglea

Mesoglea was isolated from either intact hydra or recombinants by a modification of the method of Barzansky et al. ('75). Individual specimens were placed for 30 min in a deep-well depression slide containing distilled water. After this, a detergent solution, 0.2% N-Lauroylsarcosine (Sigma) solution, was substituted for the water. The preparations were then frozen for several hr and thawed, three successive times. They were then drawn in and out of a 100-μl micropipette until all cells and cell fragments were dislodged from the mesoglea. Each mesoglea thus obtained was individually transferred to a 1 mg/ml, pH 8.8, solution of dithiothreitol (Serva) for 5 min. This was to dissolve any remaining nematocyst capsules still adhering to the mesoglea by disrupting the disulfide-linked collagen found in nematocyst capsules (Mariscal and Lenhoff, '65). How-

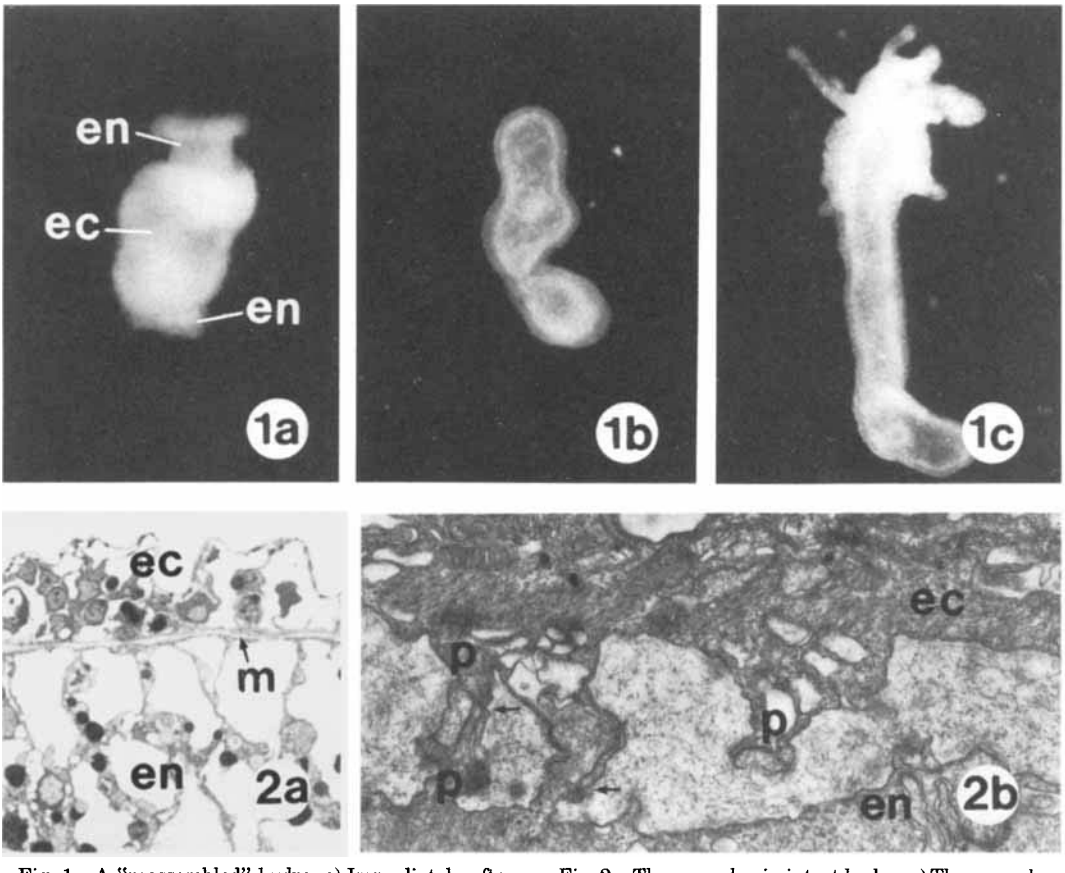


Fig. 1. A "reassembled" hydra. a) Immediately after reassembly the endoderm (en) projects from both ends of the surrounding, contracted ectoderm (ec). ×60. b) After 6–8 hr an integral hollow regenerate is produced. ×280. c) Regenerating hydra, 7 days after reassembly. ×35.

Fig. 2. The mesoglea in intact hydra. a) The mesoglea (m) is a distinct layer separating ectoderm (ec) from endoderm (en). ×525. b) In the electron microscope the mesoglea appears as a uniform meshwork of fibrils lying between the ectodermal and endodermal cells. Cellular processes (p) from the epithelial cells penetrate the mesoglea and may meet (arrows). ×12,800.

ever, in spite of this treatment, a few nematocyst capsules nearly always remained visible on the otherwise clean mesoglea. Each mesoglea was then rinsed in distilled water, individually transferred to a clean microscope slide, and allowed to dry and adhere to the glass.

Electron microscopy

Preparation of specimens for transmission electron microscopy was by standard methods that, in part, included: fixation with cold 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7,2; post-fixation in 2% osmium tetroxide in similar buffer; embedment in Spurr/Epon (1:1); uranium and lead staining. A Siemens Elmiskop 102 electron microscope was used for micrography. For scanning electron microscopy, similar glutaraldehyde fixation was followed by acetone dehydration and gold sputter-coating. A Cambridge Stereoscan electron microscope was used for micrography.

Experimental design

In order to confirm directly that both ectoderm and endoderm contribute to the formation of the mesoglea, an isotopically labeled ectoderm or endoderm was recombined with an unlabeled partner. After 72 hr, the mesoglea from such a recombination was isolated and examined autoradiographically to determine in which combination the mesoglea might be labeled. Autoradiography of semithin sections of such recombinants fixed at 4, 8, and 21 hr after reassembly was carried out

to test whether labeled compounds diffused or were transferred from one tissue to the other, thereby labeling the unlabeled partner tissue and invalidating the interpretation of the autoradiographic observations. In addition, to test whether the mesoglea became directly labeled during the exposure of donor hydra tissues to isotope or during the reassembly manipulations, and to be sure that labeled materials remained within the mesoglea for at least 72 hr, mesoglea was isolated from each of several intact labeled hydra at 1, 3, 5, 8, 24, and 72 hr after the final injection of amino acids and autoradiographically examined. In an attempt to determine the total length of time labeled amino acids remained within the mesoglea, a similar experiment was performed in which mesogleas were isolated at daily intervals for 2 weeks and autoradiographically examined.

RESULTS

Reassembly and regeneration

Valid observations using recombined tissues require that the viability of a reassembled hydra be certain. This can be determined under the dissecting microscope within 2 to 3 hr. After reassembly (Fig. 1a), ectoderm and endoderm make extensive tight contact. Ectoderm rapidly stretches to cover over endoderm. The cut ends of the epithelial tube heal over, enclosing a new gastrovascular cavity. Within 6 to 8 hr after reassembly the recombinant clearly has uniform epithelial integrity (Fig. 1b). With no opening to the exterior as yet, the gastrovascular cavity swells due to the osmotic accumulation of fluid (see Macklin, '67; Benos and Prusch, '73). Tentacles appear after about 6 days at 16°C (Fig. 1c).

It is equally as obvious, within 2 to 3 hr, if the initial reassembly manipulations are not to be successful. Endoderm does not contact ectoderm or does so only in small patches. Endoderm begins to dissociate, forming a mass of free cells and clumps of cells within the ectoderm. The ectoderm heals over and initially appears viable. However, without making good endodermal contact, the ectoderm is thrown into deep folds and bends, producing a generally asymmetrical tissue mass which dissociates after 12–24 hr.

Observations of the mesoglea in reassembled hydra

Light and electron microscopic observations made of several successfully reassembled hydra at each of several specific times after reassembly lead to the following generalized description of the mesoglea, its degradation and resynthesis.

Figure 2 demonstrates the appearance of the mesoglea in intact hydra. It appears as a distinct layer, separating and supporting the ectoderm and endoderm (Fig. 2a). Its typical ultrastructure is a homogeneous meshwork of randomly arranged fibrils, frequently invaded by processes from the cells of the two epithelial layers (Fig. 2b). Such processes often meet.

When the two epithelia are isolated, the mesoglea remains attached to the endoderm (Fig. 3; see also Epp et al., '79) although it may partially tear loose at the cut ends and perhaps fold back on itself. Thus, after reassembly of epithelia, the mesoglea is found in its normal position between the two tissues (Fig. 4a). The mesoglea appears wrinkled and varies in thickness (Fig. 4b). Cell processes are not present within the mesoglea at this time.

Three hr after reassembly (Fig. 5a,b) the ectoderm and endoderm have tightly reattached to the mesoglea. Folds have been compacted together and are fairly indistinct. Cell processes crossing the mesoglea are again not present.

By 6 to 8 hr after reassembly (Fig. 6a,b) the boundary between ectoderm and endoderm is distinct, but intervening mesoglea is difficult to find. Masses of mesoglea that have been internalized within the endoderm are obvious. These are often mixed with cellular

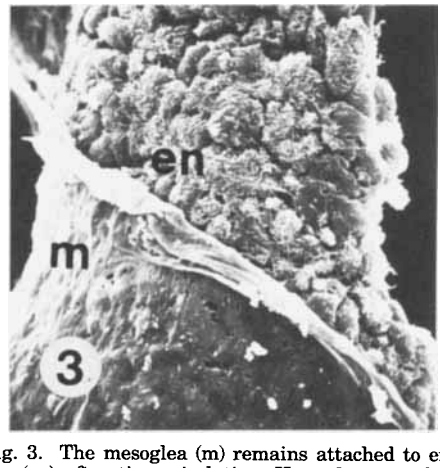


Fig. 3. The mesoglea (m) remains attached to endoderm (en) after tissue isolation. Here the mesoglea is partly torn from the endoderm and folds back on itself. ×275.

debris and seem to be in the process of degenerating.

Twelve hr after reassembly (Fig. 7a,b) new mesoglea is being synthesized between ectoderm and endoderm. It is so thin that it is not readily visible in the light microscope (Fig. 7a), but is so in electron micrographs (Fig. 7b). This new mesoglea has a trilaminar appearance. A densely-staining fibrillar layer is located centrally. A less dense, more homogeneous layer lies to each side, adjacent to the epithelial cells. Few new cellular processes penetrate the mesoglea at this time.

Twenty-four hr after reassembly (Fig. 8a,b,c) the mesoglea is a distinct structure lying between epithelia. Its thickness varies but generally is greater than that at 12 hr. The trilaminar appearance seen at 12 hr remains. Cellular processes are common within the mesoglea but are mostly associated only with the outer layers.

After 48 hr (Fig. 9) the mesoglea of reassembled hydra is similar in appearance to that of normal hydra, i.e., fibrillar, generally homogeneous in appearance, and crossed by numerous epithelial cell processes.

Autoradiographic experiments

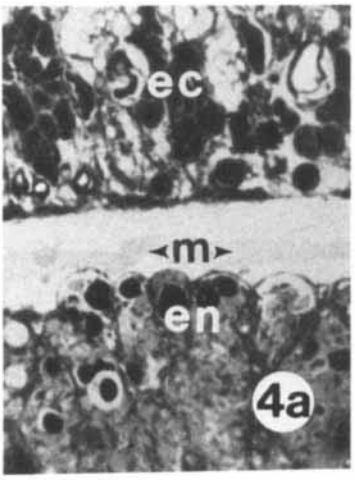
Our autoradiographic experiments confirm that both ectoderm and endoderm contribute to mesoglea formation in hydra. In each of 14 recombinations involving labeled ectoderm and unlabeled endoderm and in each of 13 opposite recombinations, mesoglea isolated after 72 hr was found to be intensely labeled. Autoradiography of semi-thin sections of such recombinants, fixed at 4, 8, and 21 hr after recombination, (two ectoderm-labeled and one endoderm-labeled at each time) always showed the originally labeled tissue alone to be radioactive. Thus the tissues considered unlabeled at the beginning of the experiment remained so. This conclusion was confirmed by another observation. As noted above, not all nematocyst capsules were removed from the mesoglea during the mesoglea isolation procedure. Nematocysts are exclusively an ectodermal product. Isorhiza and desmoneme nematocysts require about 72 hr (stenoteles take longer) to be newly created from their interstitial cell precursors (David and Gierer, '74). When a recombinant was created with labeled ectoderm and unlabeled endoderm, some isorhiza and desmoneme capsules adhering to mesoglea isolated 72 hr after injection showed intense labeling (Fig. 10a). This was probably due to proline incorporation and utilization within the ectoderm. (See e.g., Bode and Flick, '76). However, capsules were never labeled when the recombinant was originally created with labeled endoderm and unlabeled ectoderm (Fig. 10b). Thus, it is again obvious that after tissue reassembly, free amino acids do not travel from endoderm to ectoderm to be then utilized by the ectoderm.

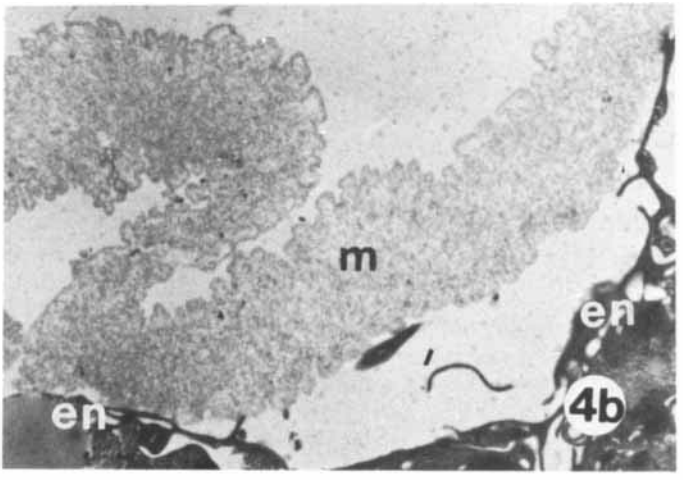
No label was found in mesoglea isolated from intact hydra at 1 and 3 hr after the final amino acid injection (Fig. 11a; 3 and 6 cases respectively). This demonstrates that mesoglea is not directly labeled during the injection or reassembly procedures. However, after 5 (Fig. 11b; 5 cases), 8 (2), 24 (5), and 72 (1) hr, mesoglea isolated from intact labeled hydra was found to be heavily labeled. This indicates that 5–6 hr are needed for newly-acquired amino acids to be incorporated within the mesoglea. Further, they are then retained in the mesoglea for a long time. Mesoglea from intact labeled hydra, isolated on a daily basis (at least 2/day) for 2 weeks after injection, clearly demonstrated label. On a purely subjective basis it appeared that the amount of label began to decrease after about 6 days, but there was no question that mesoglea was still heavily labeled at the conclusion of the experiment.

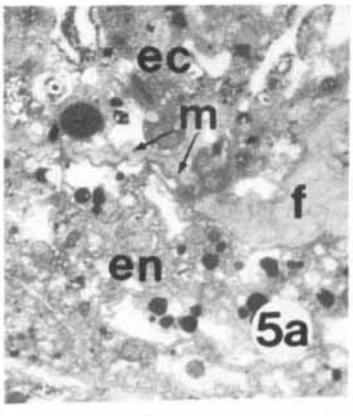
DISCUSSION

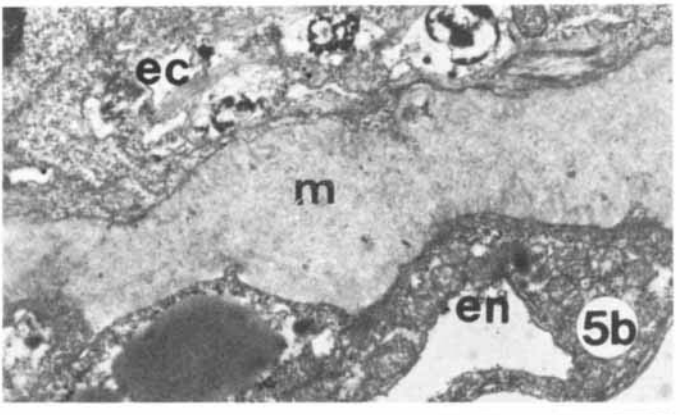
Davis et al. ('66), Davis ('73), Haynes and Burnett ('63), Lowell and Burnett ('69) and Normandin ('60) have reported regeneration of intact hydra from one or the other isolated tissue layers. However, ectoderm or endoderm, isolated by the method used here, never regenerates when cultured alone. It is developmentally interesting that reassembled tissues must rapidly and extensively reunite with the mesoglea for successful regeneration. If they do not, the integrity of the tissue begins to degenerate within a few hours. (It should be noted that dissociated cells from ectoderm and endoderm isolated by this method may participate in reaggregation and regeneration and do so as well as cells from intact hydra. Smid, unpublished; see Flick and Bode, '83.)

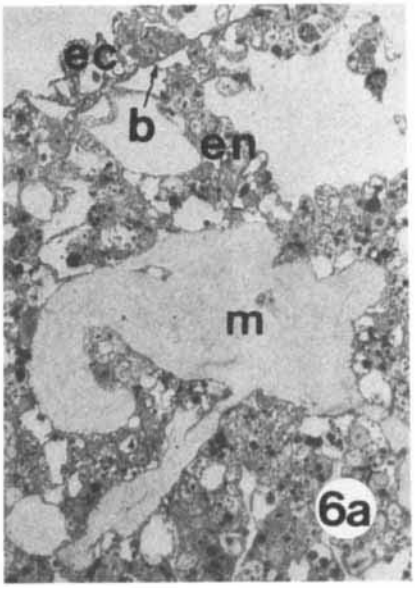
The informational nature of this physical contact, which permits successful regeneration in this system, is not known. It seems only to be the reacquisition of the mechanical support of the endodermally-attached mesoglea. This support would be reinforced by the acquisition of a hydrostatic skeleton pro-











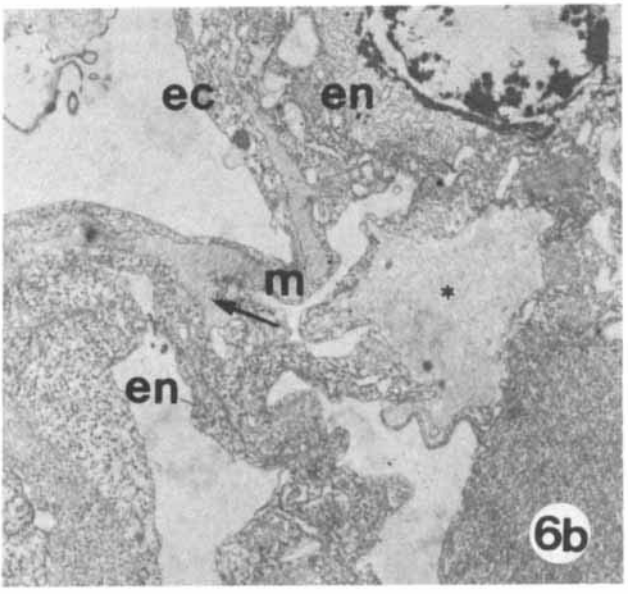


Fig. 4. Hydra tissues immediately after reassembly. a) The mesoglea (m) lies between the ectoderm (ec) and endoderm (en). ×560. b) The mesoglea is still attached to the endoderm. It is folded, variable in thickness, and its surface is wrinkled. Its elasticity and resiliency is thus obvious. ×7,500.

duced as the gastrovascular cavity swells with osmotically-accumulated fluid. Interepithelial contact across the mesoglea, through which chemical or electrical information would presumably pass, does not take place for hours after the recombinant is obviously viable.

Several observations indicate the elasticity, resiliency, and tensile strength of the mesoglea in hydra. Cellular processes that penetrate the mesoglea in intact hydra are not seen immediately after tissue isolation. Presumably they rapidly withdraw from the mesoglea, and the spaces they occupy are able to close over. The mesoglea may be stretched and torn from the endoderm during tissue isolation and may fold back upon itself. The exposed mesoglea has wrinkled edges and often appears of uneven thickness.

The initial assumption was that the "old" mesoglea in reassembled hydra would persist, and perhaps serve as a substrate for the deposition of additional "new" mesoglea during regeneration. This appears not be the case. Six to 8 hr after reassembly, mesoglea is hard to find between epithelia. Instead, mesoglea can be found internalized within the endoderm, probably to be digested there. Only after about 12 hr is mesoglea again uniformly seen between the epithelia. At this time it is extremely thin, as would be the case if it were newly synthesized. Its trilaminar appearance, dense and fibrillar in the center and light and homogeneous at both margins, suggests that the outer, non-fibrillar layers are the sites of precursor deposition from each epithelium, with fibril assembly occurring more centrally. At 24 hr the mesoglea is still layered and, as might be expected, unevenly thicker. As numerous cell processes associate mostly with the outer layers, it is likely that these processes are involved in precursor deposition. At 48 hr and later the mesoglea is of normal thickness and appearance, indicating that at least the most rapid phase of new mesoglea synthesis is over. However, mesoglea isolated from 72-hr recombinants is much flimsier than mesoglea from intact hydra. This would indicate that, in spite of its near normal appearance in the TEM, the synthesis of a biochemically mature mesoglea is not yet complete.

Davis et al. ('66) and Davis ('73) described the regeneration of an entire hydra from isolated gastrodermis. They stated that after isolation of tissues, the mesoglea was no longer present, but did not describe its fate. Lowell and Burnett ('69) reported the regeneration of an entire hydra from ectoderm. They stated that with their perfusion method, separation of ectoderm and endoderm was within the mesoglea; some mesoglea adhered to the endoderm, more to the ectoderm. However, the fate of this ectodermally bound mesoglea during subsequent regeneration was not followed. Some of the micrographs in these reports, however, indicate that early in the regeneration process there are regions with no mesoglea between epithelia. Other micrographs show extracellular spaces filled with what is variously described as mucus, cellular debris, and "elements of fibrillar material." Still other micrographs in these reports show thin regions of mesoglea between epithelia. This may represent synthesis of "new" mesoglea, although the layered appearance of the mesoglea seen in the present work is not apparent. It does seems likely, however, that in these previous studies too, "old" mesoglea was destroyed and "new" mesoglea newly synthesized. These authors did speculate that both epithelia were contributing to the synthesis of mesoglea, but did not investigate this.

That the mesoglea originates from both epithelia is certainly the case. In addition to the morphological evidence discussed above, the autoradiographic studies presented here definitively show that the mesoglea is derived from both ectoderm and endoderm. The control experiments rule out the possibility of direct contamination of the mesoglea by isotope. They also rule out the transfer of isotope from one epithelium, passive in mesoglea synthesis, to an opposite, active one.

In intact hydra, incorporation of amino acids within the mesoglea takes place in about 5 to 6 hr after initial administration of the isotope to the epithelia. Hausman and Burnett ('72) estimated similar times in their proline incorporation studies. They also believed that proline incorporated within mesoglea was initially detectable within both the fibers and the gel but later within the

Fig. 5. Reassembled tissues after 3 hr. a) The mesoglea (m) is visible as a sheet or compacted mass of folds (f) between the ectoderm (ec) and endoderm (en). ×480. b) Thickness of the mesoglea varies. Cell processes are absent from the mesoglea. Contact between mesoglea and epithelia is already tight and complete. ×6,400.

Fig. 6. Reassembled tissues after 6 to 8 hr. a) The boundary (b) between ectoderm (ec) and endoderm (en) is distinct but mesoglea (m) is not present in this location. A large mass of mesoglea is seen surrounded by endodermal cells. ×280. b) A small strip of mesoglea remains between the epithelia. One arm of this extends between endodermal cells (arrow). Nearby, mesoglea (*) is seen between endodermal cells. ×8,000.

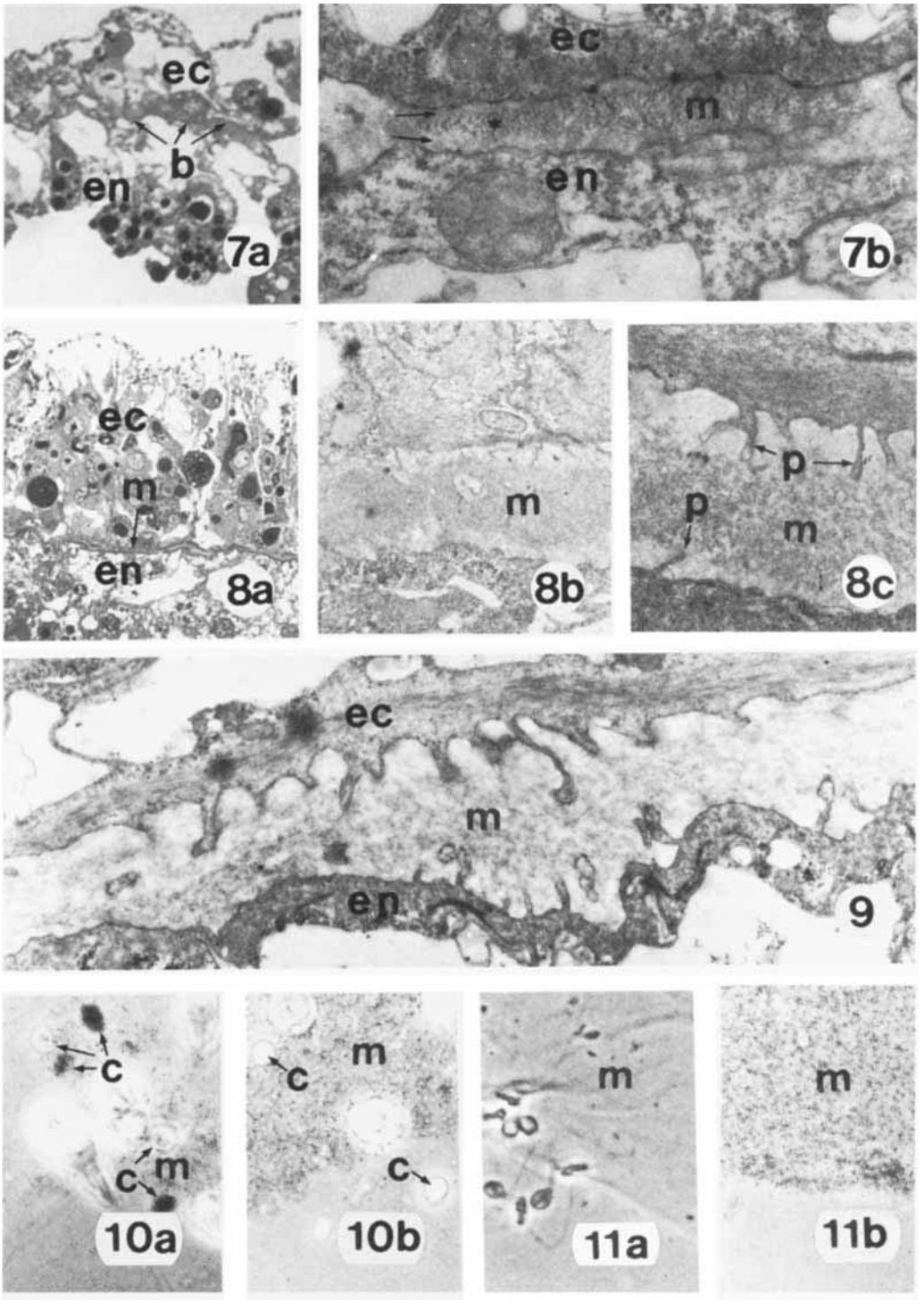


Fig. 7. Regenerating hydra 12 hr after reassembly of tissues. a) The boundary (b) between ectoderm (ec) and endoderm (en) is distinct, but mesoglea is not obvious, ×525. b) In a relatively high-power electron micrograph a thin mesoglea (m) is seen. This new mesoglea has a central densely-staining fibrillar layer (*) with less dense, homogeneous layers (arrows) to either side, adjacent to the epithelia. Cell processes within the mesoglea are very infrequent at this time. ×28,500.

fibers and not the gel. While such a distinction could not be made here, it might be that amino acids have a long residence time within the collagen fibers but continuously turn over within the amorphous gel of the mesoglea. This would account for the somewhat surprising result that amino acids are incorporated by the mesoglea within hr, but that they then remain for over 2 weeks.

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Fig. 8. Regenerating hydra 24 hr after reassembly of tissues. a) The mesoglea, variable in thickness, is obvious between the epithelia. ×475. b) The mesoglea retains the trilaminar appearance seen at 12 hr. Epithelial cell processes are associated with the homogeneous outer layers. ×22,800. c) Similar to Figure 8b. Cell processes (p) are apparent. ×37,000.

Fig. 9. Mesoglea of a regenerating hydra, 48 hr after tissue reassembly, is nearly normal in appearance (cf. Fig. 2) ×8,000.

Fig. 10. Autoradiographs of mesoglea isolated 72 hr after reassembly of tissues. ×525. a) Mesoglea isolated from a regenerate created with labeled ectoderm and unlabeled endoderm has label over the mesoglea and some capsules (c) of nematocysts. b) Mesoderm isolated from a regenerate created with labeled endoderm and unlabeled ectoderm has label over the mesoglea but not over nematocyst capsules.

Fig. 11. Autoradiographs of mesoglea isolated from intact hydra. ×525. a) Three hr after injecting tritiated amino acids no label is seen over the mesoglea. b) After 5 hr the mesoglea is heavily labeled.

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