intrahepatic accumulation, would be deleterious in a cholestatic or pre-cholestatic condition. In this respect, the capacity of the liver to hydroxylate mono- and di-hydroxy bile salts to the less toxic tri-hydroxy forms is a useful protective mechanism. The fact this metabolic pathway is more active in the rat than in man suggests that toxic effects of dihydroxy and monohydroxy bile salts could be expected at a low order of concentration in man unless this is balanced by a greater capacity to excrete the accumulated bile salts.

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Localization of substance P-like immunoreactivity in *Hydra*

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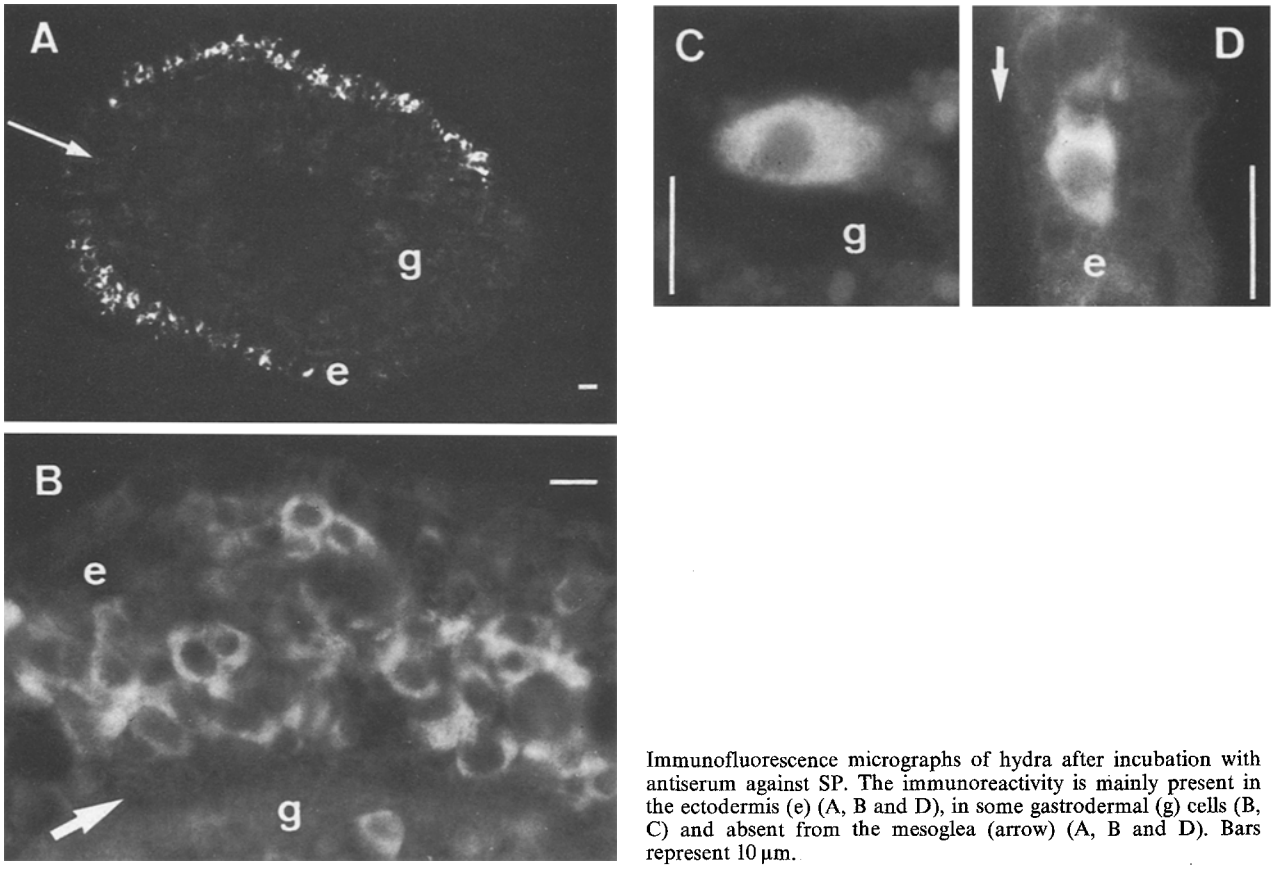
Summary. Substance P-like immunoreactivity was found in *Hydra attenuata* mainly but not exclusively in the nerve and interstitial cells, localized in the cytoplasm and on the cell surface membranes.

We have recently reported that substance P (SP) strongly stimulated hydra head regeneration[2], and the present immunological investigation was undertaken as a first attempt to establish if under normal conditions SP is detectable in this animal. SP is a small peptide mainly present in nerve[3] and intestinal cells[4] of all animals in which it has been looked for, but its presence in hydra has not yet been reported.

Materials and methods. Hydra attenuata were mass cultured in hydra medium[5] and fed once a day with *Artemia salina nauplia.* 3 sets of animals were examined. The 1st set was quick frozen in liquid nitrogen and sectioned at 8 µm with a cryo-cut microtome. The 2nd set was fixed in 4% formaldehyde in hydra medium for 3 h at 4 °C, dehydrated and embedded in paraffin wax. The 3rd set was freeze-dried, fixed with formaldehyde vapour at 60 °C and embedded in paraffin wax. To reveal the presence of SP, an indirect immunofluorescence technique[6] was employed. The sections were incubated 30 min at 37 °C in a moist chamber with anti-SP rabbit serum as 1st layer. The linkage of I^{125} labelled SP with this serum was 42% at 1:10000 serum dilution. The sections were rinsed 3×5 min with phosphate-buffered saline and reincubated with fluorescein-conjugated sheep anti-rabbit globulins (Gibco) as 2nd layer. In control experiments, the 1st layer stage was performed 1. with normal rabbit serum, 2. with anti-SP serum absorbed with newt liver powder, 3. with anti-SP serum absorbed with synthetic SP (Sigma); or 4. only the 2nd layer was applied. After rinsing 3×5 min with phosphate-buffered saline, the sections were mounted in buffered glycerin and examined in a Zeiss fluorescence microscope equipped with an HBO W lamp, a Zeiss 427902 exciting filter and a Zeiss 427903 stop filter. Photomicrographs were taken on Kodak Tri-X-Pan or Ektachrome 200 films. Counterstaining of the same sections was carried out either with hematoxylin-eosin or with silver stain. The best results were obtained with the cryostat sections.

Results and discussion. A bright green specific fluorescence appeared mainly in the ectodermis (figure, A, B, D). This SP-like immunoreactivity was present on the surface membrane and in the cytoplasm but not in the nuclei of nerve cells, interstitial cells, some large stem cells (figure I, B) and on the surface membrane of cnidoblasts. Some large gastrodermal cells also exhibited the specific fluorescence (figure, C). Most of the gastrodermis was filled with faint yellow fluorescent dots, possibly corresponding to fatty elements. The mesoglea, the central part of the cnidoblasts as well as most gastrodermal and some ectodermal cells, were devoid of fluorescence. With normal rabbit serum as 1st layer, or use of the 2nd layer alone, only a faint unspecific yellow fluorescence of the gastrodermis could be seen. Absorption of the antiserum with liver powder did not change the distribution of the specific green fluorescence, while use of the SP absorbed antiserum completely abolished it.

These results show that SP is present in *Hydra attenuata,* and moreover the SP-like immunoreactivity is not restricted to nerve cells but, to judge from the intensity of the fluorescence, SP could be abundant. The walls of most of the ectodermal cells appear rich in SP-like immunoreactivity, and this observation may be related to the previous report by Lentz et al.[7] that, where a nerve cell extension is contiguous with an ectodermal cell, it often contains neurosecretory granules situated adjacent to the plasma membrane. When isolated, these neurosecretory granules are able to induce supernumerary heads on regenerating gastric pieces of hydra[8]. Moreover they are known to contain



Immunofluorescence micrographs of hydra after incubation with antiserum against SP. The immunoreactivity is mainly present in the ectodermis (e) (A, B and D), in some gastrodermal (g) cells (B, C) and absent from the mesoglea (arrow) (A, B and D). Bars represent 10 µm.

hydra head activator (HHA)[9], a small peptide extracted from hydra which shares several common properties with SP. Both are peptides of comparable mol. wt[9,10], are present in nerve cells[3,9], in nerve vesicles of comparable size[9,11] and are able to stimulate head and tentacle regeneration in hydra[1,12]. Further experiments are in progress in order to decide if HHA and SP are the same molecule or not.

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pH and calcium concentration changes in a molluscan egg during development

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Summary. During development, the egg albumen of calcified land snail eggs becomes more and more acid, correlated directly with a constant rise in the calcium concentration of this albumen. It is suggested that the developing embryo releases some acid metabolite and the subsequent change in albumen pH aids in embryonic absorption of the $CaCO_3$ (calcite) egg shell, used for making the embryonic body shell or skeleton ($CaCO_3$ in the form of aragonite).

Recent studies have made it clear that pulmonate land snail embryos utilize egg shell calcium because not enough calcium is provided in the egg contents, i.e. egg albumen[1,2]. Some other groups of gastropods which lay their eggs on land have also been found to resorb egg shell calcium during development, as in the prosobranch *Pomacea*[3] and the soleoliferan *Veronicella*[4]. While calcium metabolism of the reproducing animal has been examined[5] no information