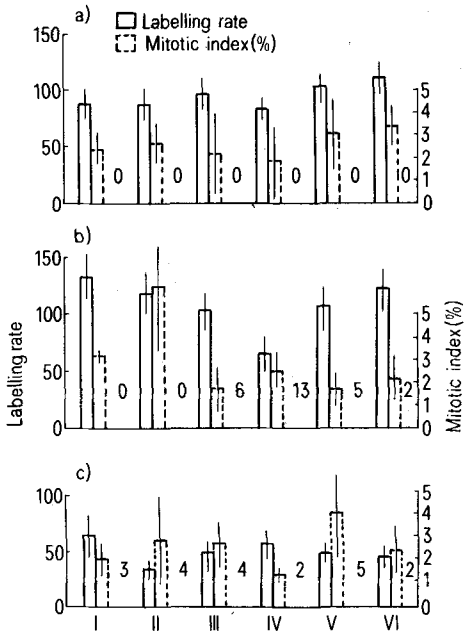

Fig. 1. DNA labelling expressed in arbitrary units, mitotic indices (%) and number of necrotic clusters (arabic numerals) observed per section in 6 (I–VI) zones of the left female (a), right female (b) and male (c) Müllerian ducts' epithelium. The values found for each zone of all ducts were pooled and the differences analyzed with the t-test. The uncertainties are SD of the mean calculated for probability $p = 0.05$. Left: the anterior zones; right: the posterior zones.

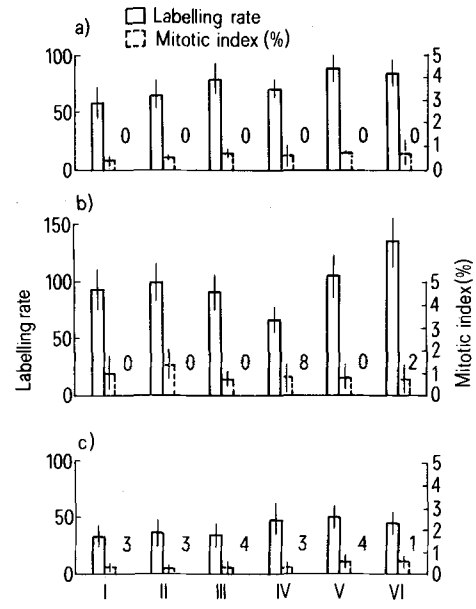

Fig. 2. DNA labelling expressed in arbitrary units, mitotic indices (%) and number of necrotic clusters (arabic numerals) observed per section in 6 (I–VI) zones of the left female (a), right female (b) and male (c) Müllerian ducts' mesenchyme. The values found for each zone of all ducts were pooled and the differences analyzed with the t-test. The uncertainties are SD of the mean calculated for probability $p = 0.05$. Left: the anterior zones; right: the posterior zones.

indices in this zone might be occasioned by mitotic arrest of the cells and mitotic death[13]. Thus, localized cell degeneration (which is not the result of mechanical injury and which is too extensive to be attributed to 'unspecific necrosis') and reduced DNA synthesis might indicate that the right female MD actually degenerates to a certain extent, starting in the more median zones.

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Synapse-like profiles in regenerating sensory nerve fibres of Herbst corpuscles

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Summary. During regeneration sequences of Herbst corpuscles, the synapse-like structures are described between pairs of non-myelinated sensory profiles. The probable significance of this unusual for sensory corpuscles finding is discussed.

It is well known that ultrastructural criteria for presumptive chemical synapses have not been elaborated in avian and mammalian mechanoreceptors[1-3]. During the electron microscopical investigation of regenerated Herbst corpuscles[4], it has been noted that one of the most characteristic feature of the new receptors is the presence of numerous non-myelinated nerve fibres in their inner core. The regenerated axons are usually encircled by long cytoplasmic processes of the Schwann receptor cells which subsequently build up the inner core of the receptors. Some of the axonal profiles lie close to each other and between them synaptoid or synapse-like contacts have been observed. The precise description of this unusual finding is an object of the present communication.

Material and methods. The 22 ducks were used as an experimental material. The animals were divided into 2 groups. The suborbital nerve branches of the 1st group were crushed, whereas the same branches of the 2nd group

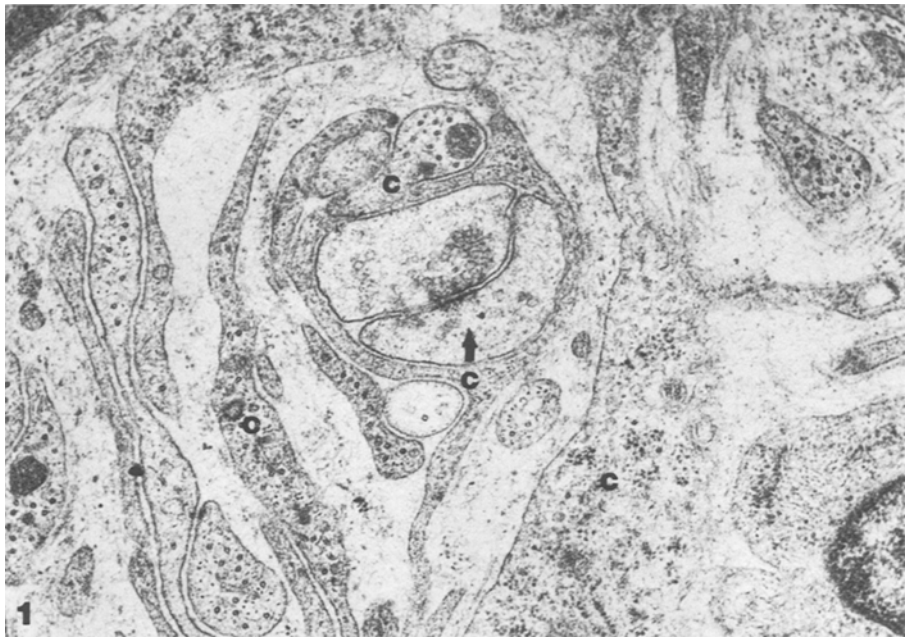
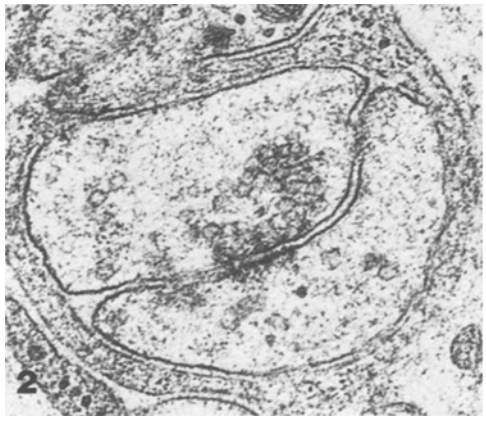


Fig. 1. Synapse-like contact (arrow) between 2 regenerated axons of Herbst corpuscle 5 weeks after nerve crushing. C – cytoplasmic processes of Schwann receptor cells. × 20,000.



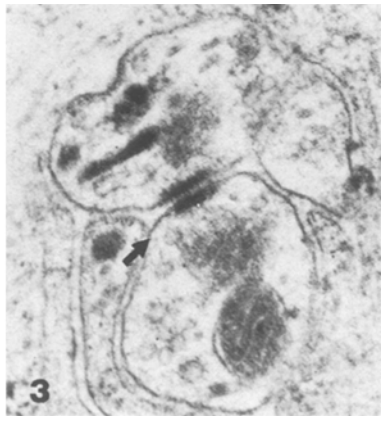


Fig. 2. Higher magnification of figure 1. × 40,000.

Fig. 3. Synapse-like contact (arrow) between 2 axons of regenerated Herbst corpuscle 7 weeks after nerve transection. × 45,000.

were transected. After both the experiments, the pieces of duck-bill skin were removed at a 30-mm away from the trauma during 3rd to the 28th weeks, one a week. The routine electron microscopic procedure was used. Electron micrographs were made on a JEM 100 B microscope.

Results. A typical feature of the closely apposed regenerated sensory axons is the occurrence of tight aggregates of vesicles at the axolemma in a configuration resembling that of synapses (figures 1 and 2). Synapse-like aggregates involve the smallest class of clear core vesicles which are comparable in appearance and size range (25–60 nm) to those commonly seen in known chemical synapses of both vertebrates[5] and invertebrates[6]. The adjacent axonal membranes show densities which extend as much as 50 nm into the axoplasm. When a synapse-like structure abuts the other axonal membrane, 'pre'- and 'post'-synaptic membranes are separated by a uniform distance of 20–25 nm for the extent of the complex, and little, if any, intermembraneous material is present. The 'pre'- but also the 'post'-synaptic grids are visible at both sides of the complex.

In other receptors, the clear core vesicles are not in close contact with the membrane densities but are oriented to them (figure 3). In these cases, the densities extend nearly 50 nm into axoplasm at both sides of the aggregate and are symmetrical in appearance. The intermembraneous material is also present. It is important to emphasize that the observed findings are established only in the first weeks of regeneration (the 4th–5th week after crushing and the 7th–8th week after transection).

Discussion. The observed unusual findings of synapse-like structures between regenerated sensory nerve branches of Herbst corpuscles and the lack of date concerning their function, requires caution to interpret their presence.

Axo-axonal synapse-like structures have been observed between neurites of regenerated motor nerves of crayfish[7], and rarely in invertebrate sensory nerves of a spider[8]. In our observations, the pre-synaptic side meets the morphological criteria valid for chemical synapses, but the post-synaptic element has the same density and structure as the pre-synaptic. The 2nd argument, which is at odds with the presumption that the observed profiles are chemical synapses, is the transitory character of these structures which appear only early in the regeneration sequence. Finally, the axo-axonal junctions are between the pairs of regenerating sensory fibres and never between proximal and distal axon stumps.

The great number of new axons perhaps reveals the tendency of proximal stumps to make contacts with appropriate receptor cell structures during re-innervation. The axonal profiles can be derived from one or more sensory

cells. In both cases, the observed contacts are suggestive of movement of substances between 2 conventional structures. Perhaps, like synapses, they are sites of release of substances which may serve directly for the morphogenesis and maintain the receptor cell elements during early stages of regeneration.

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Immunochemical identification of vitellogenin in the serum of the newt *Triturus cristatus*[1]

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Summary. The hematic yolk precursor - vitellogenin - has been identified immunochemically in the serum of estrogenized females of the newt *Triturus cristatus* by employing an antiserum prepared against yolk proteins.

Amphibian oocytes undergo vitellogenesis by virtue of their capability to pinocytose large quantities of a hematic yolk precursor generally referred to as vitellogenin[2,3]. The release into the blood stream of this precursor molecule is subsequent to its synthesis which is known to take place in the liver[4]. Available evidence indicates that both the hepatic synthesis and the oocyte uptake of vitellogenin are hormone-controlled processes[5]. It is in fact well established that while estrogens enhance the synthesis of vitellogenin in the liver but inhibit its uptake by the oocyte[6-8], gonadotropin appears to have a stimulating effect on both processes[9]. Much of the present knowledge on vitellogenesis of amphibians relies on experimental studies carried out with anurans mainly by using chromatographic techniques[10]. Not so much information is available to date on vitellogenesis in urodeles, and this led us to examine this process in newts and in particular to verify the feasibility of an immunological approach to this kind of study.

Material and methods. Specimens of the species *Triturus cristatus carnifex* (Laurenti, 1768) were collected in the surroundings of Pisa and reared in laboratory conditions only for the period of experimental treatment. Blood samples were obtained by heart puncture of females estrogenized 24, 48 or 72 h before. After clotting, newt serum was analyzed by 7% native acrylamide gel electrophoresis[11]. Aliquots of newt serum were also treated with 2% sodium dodecyl sulphate in 0.0625 M Tris-HCl buffer at pH 6.8 (SDS sample buffer) and analyzed by 15% acrylamide SDS electrophoresis[12]. Protein fractions were evidenced by staining the acrylamide gels with 1% Coomassie blue in 10% alcohol: 45% acetic acid or in 1% Ponceau red in 12% trichloroacetic acid (TCA). Protein fractions in newt serum were identified by comparison with human serum and termed according to the known terminology. Phosphoproteins were selectively stained according to the procedure of Green et al.[13] who employed the cationic carbocyanine dye 1-ethyl-2-[3(1-ethylnaphtho[1.2d]thiazolin-2-ylidene)-2-methyl-propenyl]naphthol[1.2d]thiazolium bromide (ETB). Yolk platelets were extracted from isolated vitellogenic oocytes by means of the polyvinylpyrrolidon (PVP)-sucrose gradient method of Wallace and Karasaki[14]. To detect vitellogenin in newt serum and to compare it with yolk extracts, antisera against whole newt serum (AWS) and against yolk proteins (AYP) were prepared in rabbits[15]. Newt serum and yolk proteins were tested with respective antisera by agar double immunodiffusion technique[16], immunoelectrophoresis[17] and crossed immunoelectrophoresis[18].

Results and discussion. When serum of estrogenized female was analyzed by native acrylamide gel electrophoresis, at least 8 different protein fractions could be evidenced (figure 1). As a 1st criteria to identify vitellogenin amongst these protein fractions, we took advantage of knowledge of its high protein phosphorous content[19]. Accordingly, a few acrylamide gels were stained for phosphoproteins with the cationic carbocyanine dye ETB. By this staining technique, only 2 protein fractions could be differentiated in the alpha-1 and beta-2 regions of the gel, respectively. To ascertain which of the 2 ETB-positive protein fractions could be related to vitellogenin, a few 7% acrylamide were sliced by using as a reference a co-electrophoresed stained acrylamide gel. Each acrylamide slice was then treated with SDS sample buffer, inserted into an SDS slab 15% acrylamide gel and subjected to overnight electrophoresis. The results of this analysis showed that the beta-2 protein fraction exhibits a mol.weight higher than that of the alpha-1 fraction and lies within the range of that expected for an amphibian vitellogenin. Although phosphoprotein staining and mol.weight determination may provide indications as to the presence of vitellogenin in the beta-2 region, they do not prove it. To overcome these limitations, vitellogenin antigenity was taken as a reliable test for its identification in newt serum. When newt female serum was

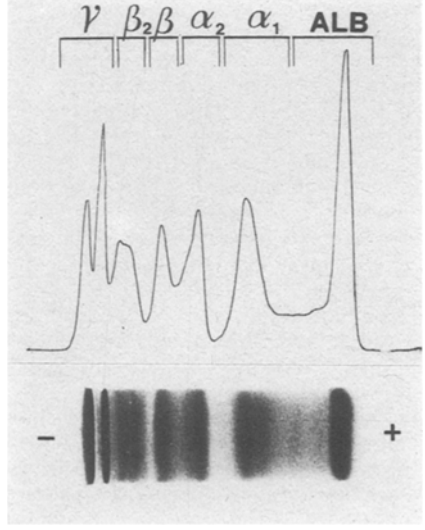

Fig. 1. Native acrylamide gel electrophoresis of female newt serum stained with Ponceau red and analyzed on a Cellomatic densitometer. γ, β_2, β, α_2, α_1, and ALB refer to the various protein fractions identified in the newt serum.