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

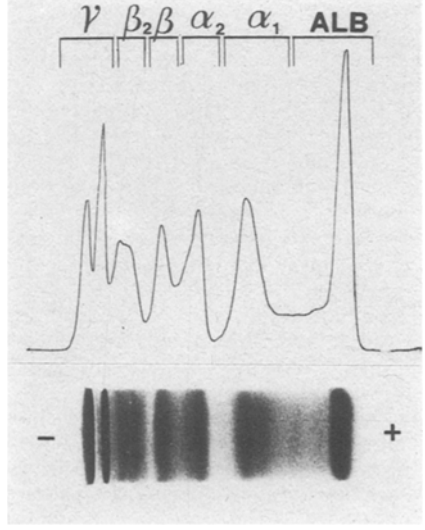

Fig. 1. Native acrylamide gel electrophoresis of female newt serum stained with Ponceau red and analyzed on a Cellomatic densitometer. γ, β_2, β, α_2, α_1, and ALB refer to the various protein fractions identified in the newt serum.

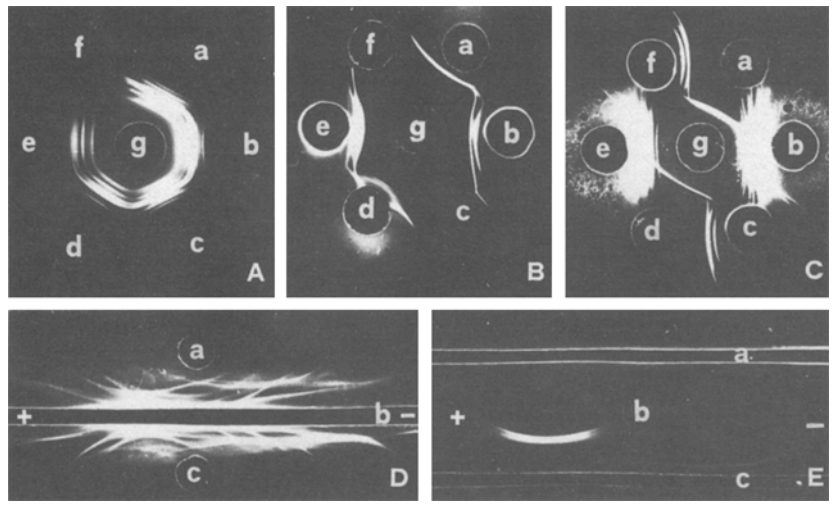

Fig. 2. *A* Double immunodiffusion plate showing specific antigens detected by AWS (g) in female newt serum employed at various concentrations (a: undiluted newt serum; b: 75% newt serum diluted in 0.15 M phosphate buffer saline (PBS); c: 60% newt serum diluted in PBS; d: 50% newt serum diluted in PBS; e: 25% newt serum diluted in PBS; f: bovine serum albumin (BSA) at 1 mg/ml. *B* Double immunodiffusion plate showing specific antigens in yolk extracts (b and e) and estrogenized female newt serum (a and d) as detected by AYP (g). c and f: BSA at 1 mg/ml. *C* Immunodiffusion plate showing identity reaction among yolk antigens (c and f) as detected by AYP (a and d) and vitellogenin from estrogenized female newt serum (g) as detected by AYP (b and e). Note that vitellogenin gives also rise to an identity reaction with one of the antigens detected by AWS in female newt serum. *D* Immunoelectrophoretic plate showing a number of antigens in estrogenized newt female serum (a and c) as detected by the specific antiserum AWS (b). *E* Immunoelectrophoretic plate showing the vitellogenin arc identified by AYP (c) in estrogenized female newt serum (b). (a) was filled with non-immunized rabbit serum.

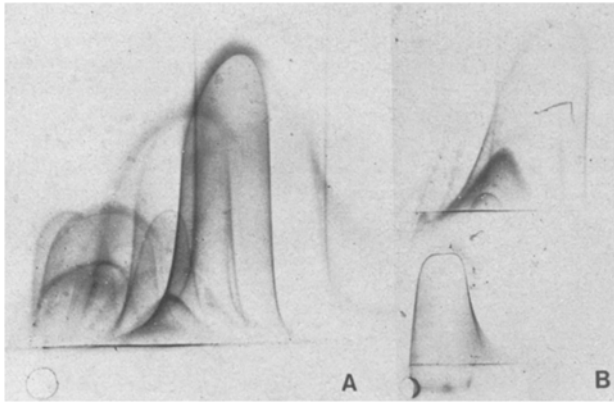

Fig. 3. *A* Crossed immunoelectrophoresis of newt serum from estrogenized females electrophoresed against AWS at a dilution of 60 µl/6 ml of 1% agarose in veronal sodium buffer. Electrophoresis was run for 2 h at 25 mA/100 V for the 1st dimension and then overnight at 7.5 mA/30 V for the 2nd dimension. *B* Crossed immunoelectrophoresis of newt serum from estrogenized females electrophoresed against a composite 1% agarose gel containing AYP (lower gel) and AWS (upper gel). Electrophoretic conditions were as those reported in *A*.

examined by a double immunodiffusion test in 1% agar against AWS, at most 6 or 7 precipitation arcs could be observed (figure 2, A). On the other hand, when this serum was made to react with AYP, only 1 precipitation arc could be revealed (figure 2, B). The intensity of this reaction was somewhat related to the length of the estrogen treatment given to the newt.

When yolk proteins were made to react with AYP, 2 precipitation arcs could normally be observed and both of them gave rise to an identity reaction with the vitellogenin arc identified by AYP in newt serum (figure 2, B and C). These findings are in line with the hypothesis that the hematic yolk precursor - vitellogenin - consists, in *Triturus* as in *Xenopus*, of a unique molecular form. This could also be demonstrated by immunoelectrophoresis in agar gels. By this technique only 1 precipitation arc could be revealed in the serum when tested against AYP, indicating that vitellogenin is also electrophoretically homogeneous (figure 2, E). This is probably one of the many antigens revealed by AWS in estrogenized female newt serum (figure 2, D).

In order to identify vitellogenin among the various serum antigens, the immunodiffusion plate shown in figure 2 C was set up. In this plate, 1 of the precipitation arcs identified by AWS in the serum gives rise to an identity reaction with the one identified by AYP. The identity of 1 serum antigen with vitellogenin could be better appreciated by analysis of the newt female serum in crossed immunoelectrophoresis. As shown in figure 3 B, the vitellogenin antigen in the serum determines the formation of a distinct precipitation arc in a gel containing AYP. Other antigens are not impeded in their migration by the presence of the AYP and so may interact with their specific antibodies in a gel containing AWS. The result of this interaction is an immunoelectrophoretic pattern comparable to that of figure 3 A. The electrophoretic position yielded by vitellogenin in this type of gel is likely to correspond to the one exhibited by the phospho-protein-stained band present in the beta-2 region of the 7% acrylamide gels.

So far vitellogenin identification has been achieved through detection of its high phosphorous content which can be used as its specific marker[19,20]. Although preliminary, the present results show that vitellogenin may also be identified immunochemically in various types of electrophoretic analysis. This kind of approach may therefore prove useful for testing vitellogenin presence during its chromatographic isolation from newt serum.

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Experimental desquamation of intestinal epithelium for in vivo studies of regeneration

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Summary. Complete removal of villous as well as upper-crypt epithelium was achieved in vivo by vibration of an intestinal segment using tetraphenylboron sodium (TPB) as a disassociation agent.

Intestinal epithelium, being a rapidly proliferating and differentiating cell population, is a popular model for studies on regeneration. The common disadvantage of various experimental models in studies of intestinal epithelium regeneration[2-8] is either the damage of the underlying parts of the intestinal wall (ischaemia, thermic, chemical, or mechanical injury) or their influence on the whole body (radiation injury). Thus, most of these methods, when applied to the small intestine of animals, seem to be insufficiently accurate in evaluating the cellular events occurring in the course of the intestinal epithelium regeneration process.

The purpose of the present work was to adapt the in vitro Harrison and Webster vibration method[9] of cell isolation from everted segments of small intestine for studies on the regeneration of small intestinal epithelium. We expected that this method would have a less harmful effect on the lamina propria and other parts of the intestinal wall, thus allowing more precise and impartial observation of some phenomena occurring in the course of the regeneration process.

Materials and methods. 24 male Wistar strain rats with a weight range of 180–250 g were used. All animals were starved for 24 h and were given free access to water before the final experiment. The animals were anesthetised by i.p. injection of 3.6% chloral hydrate. The distal ileum was exposed after an abdominal wall incision in the left side of the body. The 2 ligatures were tied across the exposed intestine; the 1st one at 5 cm and the 2nd at 15 cm from the ileocaecal junction. The 10-cm-long segment of the ileum between the 2 ligatures formed a sac into which 1.5 - 2.0 ml of isolation medium was injected to the point of moderate dilatation of the intestinal wall. The sac with intact mesenteric vessels was placed in a vibrating holder (figure 1). The source of vibration was the Predom-Zelmer vibrating apparatus (100 Hz, amplitude 0.5 mm). The segments were vibrated for 15 min in all the experimental groups. The ileal sacs of 5 rats (group I) were injected with 0.15 M NaCl, 9 rats (group II) were injected with 5 mM EDTA + 0.14 M NaCl + 0.05 M sucrose, and 6 rats (group III) with 3 mM tetraphenylboron sodium (TPB) + 0.14 M NaCl + 0.05 M sucrose. Samples of vibrated segments of ileum and the control material taken 10 cm proximally were excised for histological examination immediately after vibration. Specimens were fixed in a mixture of 2.5% glutaraldehyde and 2.5% formaldehyde and embedded in epon. Semithin sections (1 µm) were stained with toluidine blue.

Results. The morphological appearance of segments vibrated with a medium consisting only of saline (group I) was insignificantly changed as compared with the controls. The mucosa had maintained its integrity and only the villi were somewhat shorter but of the same finger-like appearance. Epithelium of the lower parts of the crypts showed enhanced basophilia as compared to the controls; moderate

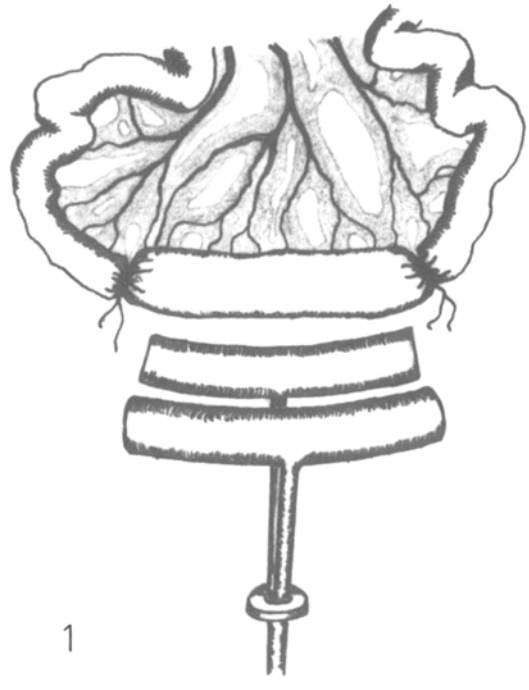

Fig. 1. Schematic drawing showing the vibrating holder and the intestinal loop with 2 ligatures.