weight, 10 minutes at 37°C). The purified lectin exhibited hemagglutinating activity at a minimum concentration of 10 μg/ml. This activity was inhibited by Man-6-P and by the other sugars shown in Table 1. These preliminary results, therefore, suggest that the lectin has a subunit of 56K.

Lectin activation by limited proteolysis is, as far as we know, a novel phenomenon in lectin research. It can be explained in two ways. One is by a high association constant between lectin and endogenous glycoconjugates, which should produce a concentration of free hemagglutinin below the detection limit of the hemagglutination assay. Trypsinization of the lectin would result in hemagglutination activity because of preferential digestion of the ligand, which, once digested, could display a lesser affinity to the lectin, with a consequent increase in the concentration of free hemagglutinin that would now be detected by hemagglutination (*14*). The other explanation for the lectin activation is that the *Giardia* lectin is in the form of an inactive hemagglutinin (prolectin), which, on proteolysis, will generate active lectin by exposing more than one sugar-binding site to the environment.

Since trypsin is abundant in the segment of the small intestine where *Giardia* thrives, it may be involved in the in situ activation of the parasite lectin. Once activated, the lectin may react with the small intestine mucosa to produce parasite attachment. This is in accordance with the observation that the activated lectin reacted with enterocytes in vitro. The activation of the *Giardia* lectin by a host protease could be similar to a mechanism in myxovirus, in which specific proteolytic cleavage of a surface glycoprotein is essential to render the virion infectious (*15*), and to a mechanism in *Trypanosoma cruzi*, in which proteolysis of surface membrane proteins appears to enhance infection of host cells in vitro (*16, 17*).

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Living *Nautilus* Embryos: Preliminary Observations

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***Nautilus*, long recognized as the most primitive living cephalopod, provides insight into molluscan evolution. Despite many attempts, embryos have not been observed until now. This report details the surface morphology and extraembryonic circulatory pattern. It was found that development, as in other extant cephalopods, is direct, without larval stages. There appears to be no embryonic protoconch associated with shell ontogeny.**

IN 1832 OWEN (*1*) DESCRIBED *NAUTILUS* and noted the importance of finding the embryos (*2–5*). Many questions concerning the phyletic relations in the Mollusca could be resolved by an understanding of the embryology of this most primitive living cephalopod (*6–10*). Among the many attempts to obtain embryos was that of Willey (*11, 12*) in the late 1800's. He described infertile egg capsules but did not obtain embryos. Haven (*13, 14*) kept adult animals in submerged cages for 1 year, but eggs were not laid. Mikami and Okutani (*15*) and Mikami *et al.* (*16*) described copulation and egg deposition in captive *Nautilus*, as did Carlson (*17*), but embryos did not develop. Until 4 March 1985 none of the egg capsules that we had opened contained living embryos, although one contained an embryonic shell. We report here egg deposition in *Nautilus* and preliminary observations of the living embryos.

Eleven adult *Nautilus* (seven females) obtained in July 1982 off Palau (7.2°N, 134.3°E) were maintained in two tanks. The flow from a saltwater well was 50 liter/hour and the temperature fluctuated from 15°C (daytime) to 24°C (nighttime), simulating natural conditions (*18*). After 5 months, egg laying began and was nearly continuous for 30 months. Egg capsules were separated from the adults (who tended to eat them) and placed in a tank at 21°C. By February 1984, 70 egg capsules had been examined, one of which contained a shell. Thirty-one more egg capsules laid between November 1984 and May 1985 were opened and five living embryos and one shell were found.

The egg capsules, deposited on vertical surfaces of the aquarium or on rocks, conformed to the contour of the substrate. The outer layer of the capsule was open to the sea by several channels, each a few millimeters in diameter. A teardrop-shaped inner capsule surrounded an ovate mass of semifluid yolk about 1.4 by 2 cm in diameter (Fig. 1A). Within the apex of the inner capsule was a space where the embryo developed (*12, 15*). Infertile eggs and blastoderm-stage embryos had a definite, semitransparent chorion that was not obvious in the older embryos, and there was whitish egg jelly between the chorion and the inner capsule. Two embryos were in the blastoderm stage (one 14 days old), one was in an early organogenetic stage, and two had shells covering the visceral mass, which is contained in the mantle cavity in the adult.

The blastoderm (Fig. 1B) was 6.7 mm in diameter and bulged outward from the contour of the yolk mass; it appeared to be constrained by the chorion. At its edge was a circle of large, white, triangular cells similar to the blastocones described for other cephalopod embryos (*6*). Inside this was a region of relatively uniform cells. Internal to this were bilaterally placed cells of several thicknesses, but it was not possible to identify organ primordia at ×100 magnification. Symmetrical folds were observed in the blastoderm, but these may have been caused by

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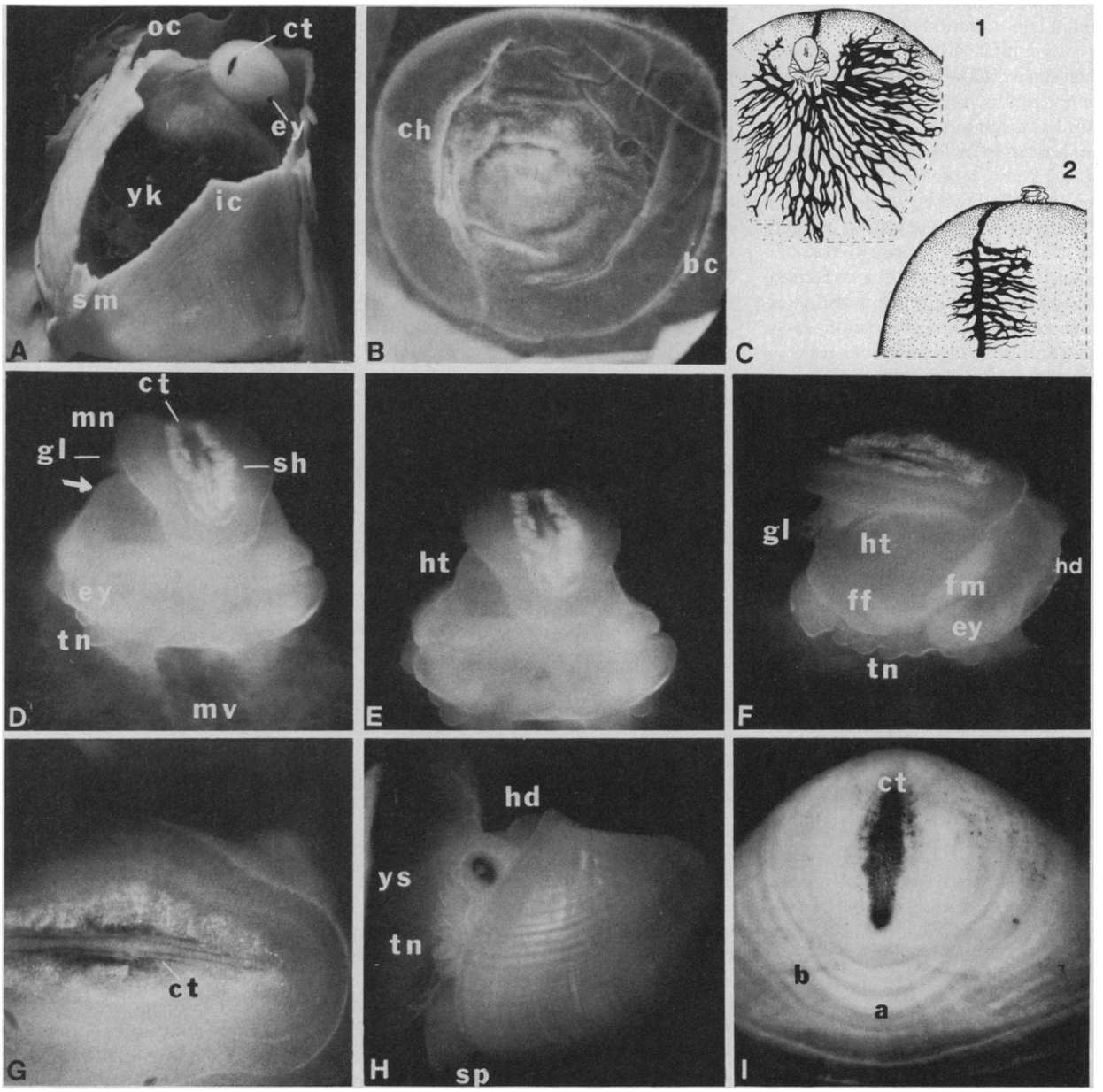

Fig. 1. (A) *Nautilus* embryo in situ in the egg capsule. The outer capsule (oc) has mostly been removed and the inner capsule (ic) has been opened along a seam (sm) to show the embryo with the elongate cicatrix (ct) on its shell. One eye (ey) is evident. The large yolk mass (yk) is enclosed in an external yolk sac, which is actively contractile. Note the striated pattern on the inner egg capsule (magnification, ~ ×2.5). (B) Large triangular cells (blastocones, bc) at the margin of the blastoderm. The chorion (ch) has been partially removed. The central region is bilaterally symmetrical and organ primordia are appearing but cannot be distinguished. Between the central organogenic area and the blastocone margin is the future external yolk sac region (~ ×8). (C) Camera lucida drawings of the extraembryonic circulatory system of the embryo shown in (D) to (G). In drawing 1, multiple vessels branch out from the future dorsal surface and ramify over the external yolk sac surface. One large, irregular vessel is evident on the future ventral surface (drawing 2), and probably is the venous return. It is paralleled by two large muscle bands. (D) Circulatory movements of the embryo during early embryogenesis. The area indicated by the arrow contracts rhythmically and probably is the primordial branchial heart that circulates the embryonic blood through the gills and to the external yolk sac. The cicatrix primordium, forming shell (sh), eye, tentacle buds (tn), mantle (mn), median vessel (mv), and gill primordium (gl), are labeled (~ ×17). (E) Contraction of the primordial branchial heart region (ht), presumably forcing the fluid it contained through the gill primordium and into the extraembryonic circulation (~ ×17). (F) Side and top view of the embryo at early organogenesis. The mantle containing the developing shell is prominent. The gills develop as two pairs, with the outermost being most advanced and containing a developing blood vessel. The gill filaments are just beginning to appear at the base of this gill. The funnel folds (ff) and associated musculature (fm) are evident between the primordial heart (ht) and the eye complex. The tentacle buds (tn) appear as a series of protrusions at the junction of the embryonic body and yolk sac. The future hood (hd) appears at the midline, behind the eye primordia (~ ×17). (G) The shell developing inside the mantle on either side of the bands of black pigment of the primordial cicatrix. The reflective white spots are the forming crystals of the shell (~ ×35). (H) Older embryo, the shell envelopes the viscera while the siphon (sp), hood, eyes, and arms (tn) emerge between the shell and external yolk sac (ys). The embryo is active and rhythmic respiratory movements are evident. The eye is red, the cirri are developing the digital tentacles, and the siphon opens and closes in response to exhalant flow. This embryo is oriented in the adult functional position (~ ×8). (I) Cicatrix region in the older embryo, composed of an oval of black pigment that grades into the shell material. Two whitish regions (a and b), which appear to be thicker shell, are evident. These regions correspond to the future position of the first and second septa (~ ×17).

leakage of yolk at the edge of the blastoderm.

The embryo in early organogenesis showed typical cephalopod features with modifications due to the massive amount of yolk. Most striking was the presence of an extraembryonic circulatory pattern with vessels ramifying out from the future dorsal surface in a somewhat symmetrical pattern (Fig. 1C). A single large median vessel ran over the yolk surface to the future ventral surface of the embryo. It was connected to perpendicular branches and was irregular in thickness and outline. Two bands of muscle paralleled the vessel (Fig. 1D) and eventually dissipated over the yolk sac surface. It is likely that this median vessel returns blood to the embryonic body.

The living embryos had lateral areas of rhythmic pulsatile contraction between the mantle primordium and the primordial funnel musculature. In other cephalopod embryos, this area would form the vena cava and branchial hearts. Figure 1 shows this region in expanded (Fig. 1D) and contracted (Fig. 1E) condition (average interval, 14 seconds; range, 9 to 18 seconds; 22°C). Peristaltic contraction of the yolk sac also occurred. The elongate mantle with its developing shell was prominent (Fig. 1F), and at the center there were multiple lines of black pigment paralleling the midline of the shell, which were undoubtedly the primordium of the cicatrix. Hyatt (*19, 20*) believed that the cicatrix represented the scar where a primordial embryonic shell had been lost later in development, but no evidence of any such "protoconch" was observed.

On either side of the cicatrix were arrays of reflective white particles perpendicular to the axis of the cicatrix. This was, apparently, the beginning of shell primordium. It was covered with a clear layer not visible in the photomicrographs. On the future ventral surface of the adult were two pairs of gill primordia; the distal ones were considerably more developed than the proximal pair and had a central blood vessel (Fig. 1F).

The proximal gills moved in synchrony with the contraction of the presumed embryonic heart. A slight vertical thickening of the tissue between the proximal gills suggests the hind gut primordium. Below the heart region the rudiment of the funnel and funnel musculature was evident as a thickened band of tissue elevated from the embryo (compare Fig. 1, D to F). A thin band connected it to the mantle primordium. Below and ventral to the gills, in a broad band at the junction of the embryonic body and the yolk sac, was a mass of tissue faintly divided into two groups of three on either side of the midline. On either side of the band, and running along the margin of the yolk sac, two convoluted groups of tentacle primordia were divided into four equal sized projections. Directly behind them and below the eye primordium complex were two more projections, unequal in size: the ocular tentacle primordia. On the midline there was a projecting ledge of tissue presumed to be the rudiment of the hood. The eye primordia were located on either side of the future hood, above the unequal sized tentacle buds and near the funnel musculature. At this stage the eye had completed invagination but the optic vesicle was still open. Between the optic vesicle and the hood rudiment was a thickened mass of tissue possibly associated with the eye. Since these tissue masses and the optic vesicles were in juxtaposition and formed a conjoined curved mass, they are referred to here as the eye complex.

In the two older embryos the shell had developed to cover the viscera approximately to the adult position (Fig. 1H). The eye, the optic stalk, rhinophore, and ocular tentacles had developed into a distinct unit, the eye complex. The eye was a red, curved cup with a flattened distal surface borne on the optic stalk. The pupil was open and was centered over the pigmented retina. The sheaths of the ocular tentacles appeared hollow and probably contained developing circi. Many more tentacle buds were evident at this stage, and they seemed to be in rows, suggesting that at least one other band of tentacle primordia arose above the obvious ones of the earlier embryo. The digital tentacle buds were clustered in two bilobed groups between the eye and funnel and resembled the ocular tentacles. Movement of the tentacle buds was not apparent. The funnel was large, in continuous motion, and its edges slid over one another in synchrony with the respiratory movements. The embryo contracted, pulling the shell and visceral mass closer to the yolk sac once every 2.6 seconds on average. During the contraction phase the funnel was expanded and extended and pointed in different directions. Sometimes, during the inhalant phase, it retracted almost completely into the shell.

When the embryo was disturbed by vibration, the shell pulled sharply toward the yolk and the funnel retracted. The hood at this stage was a shelf-like bulge in the future dorsal area and protruded slightly from the shell (Fig. 1G).

In the external yolk sac the extraembryonic circulatory pattern was more interconnected and complex. The vessels varied in size, but preliminary sections did not show capillary-sized vessels. In some places vessels could be seen passing below others. It was not possible to see the direction of flow in these vessels. The animal third of the yolk sac appeared muscular, and on irregular occasions sharp contractions suddenly circumferentially constricted the yolk sac. It took up to 1 minute for the sac to return to its contour. Occasionally, contractions of localized areas lasting several seconds occurred near the embryo, but did not displace it. The whole embryo, including the yolk sac, rotated within the egg capsule once every 21 minutes on average ($n = 4$).

The shell was covered with a uniform transparent layer, presumably the periostracum. A prominent pattern of longitudinal and latitudinal striations on the outer surface of the shell could be traced back to the cicatrix. This pattern of striation extended beyond the edge of the calcified shell into the periostracum. Shell crystals appeared first at the junctions of the crossed striations (Fig. 1I). The cicatrix was an elongate oval of black pigment at the midline that peripherally broke into an irregular group of spots which, in some places, corresponded to the longitudinal striations (Fig. 1I). There did not appear to be any specialized structure on the cicatrix. When viewed with oblique lighting, two whitish areas were apparent, one that followed the outline of the cicatrix, about 0.7 mm from it, and another larger one that was shaped like a blunted heart.

From this description of limited material a few interesting features of *Nautilus* ontogeny can be discussed. Development is cephalopod-like, with a blastocone-edged blastoderm spreading on a large fluid yolk mass. The placement of organ primordia is comparable to that in other cephalopods (*6*) when the size and yolk mass are considered. The comparatively early development of the shell and mantle is not surprising in an animal with a large external shell; nor is the early presence of a large circulatory system, considering the large mass of yolk and the size of the embryo. The elaborate extraembryonic circulatory system may be unique in invertebrates, however. In previously described cephalopods the external yolk sac contains a simple hemal space traversed by muscle bands rather than a system of well-developed vessels which apparently diffuse and recoalesce to return to the embryonic body. The cilia, movements in the egg capsule, and contractions of the musculature of the external yolk sac suggest a respiratory as well as digestive function for this organ. The lack of any specialized external structure associated with the cicatrix suggests that a protoconch is not associated with shell formation. The black material of the cicatrix and at the site of apparent repair, together with the data on shell regeneration presented elsewhere (*21*), suggest that the "black organic material" is associated with early shell deposition, although modeling of the

shell pattern by periostracum is obvious. The development of the funnel, tentacles, and hood will require further analysis before conclusions can be drawn regarding the relation of these organs to the head and foot.

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In Vivo Competition Between a Metallothionein Regulatory Element and the SV40 Enhancer

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The human metallothionein-II$_A$ (hMT-II$_A$) gene contains an enhancer element within its 5′ regulatory region. This enhancer element can compete with the SV40 enhancer for one or more cellular factors in vivo. The competition between the two elements is modulated by cadmium, an inducer of hMT-II$_A$ transcription. The data presented are consistent with a model in which heavy metal ions control the ability of the hMT-II$_A$ enhancer to bind a positive factor, leading to increased transcription. The same factor is required for maximal activity of the SV40 enhancer, which suggests that viruses utilize factors that have a normal role in cellular gene expression to control their own genes.

EXPRESSION OF EUKARYOTIC GENES transcribed by RNA polymerase II is controlled by a variety of *cis*-acting genetic elements, including promoters (*1*), enhancers (*2, 3*), and responsive elements (*4–6*). These *cis*-acting elements are thought to serve as binding sites for *trans*-acting regulatory proteins. However, with few exceptions, including T antigen (*7*), heat shock transcription factor (*8*), SP1 (*9*), and the glucocorticoid (*6, 10*) and progesterone (*11*) hormone receptors, none of these factors or their interactions with DNA have been characterized.

The human metallothionein-II$_A$ (hMT-II$_A$) gene (*12*) can be induced by heavy metal ions and glucocorticoid hormones. This induction (and control of the basal level of the gene's expression) are regulated by elements within the 5′ flanking region of the hMT-II$_A$ gene (*6*). Similar elements have also been described for the mouse (m)MT-I gene (*13*). These regulatory elements can activate heterologous promoters in a distance-independent manner (*6, 14*), a property shared with enhancer elements (*2*). We have characterized an enhancer element, present within the hMT-II$_A$ 5′ regulatory region (*15*), whose activity is further increased in the presence of heavy metal ions such as cadmium (Cd^{2+}) (*16*).

Recently, Scholer and Gruss (*17*) developed a novel approach that allows further insight into the mechanisms that control gene expression, in the absence of detailed knowledge of the regulatory factors involved. By means of an in vivo assay, they have demonstrated competition between enhancer-containing molecules derived from SV40 and murine sarcoma virus for cellular targets. Tissue-specific competition has been demonstrated between immunoglobulin and SV40 enhancers (*18*). All of these enhancers contain a common sequence known as the "core" that is important for their function (*19*). We now show that regulatory elements of the hMT-II$_A$ gene are capable of competing with the enhancer element of SV40 (and vice versa) for a common cellular target and that the competition is modulated by Cd^{2+}.

To understand how Cd^{2+} regulates the activity of the enhancer element we have used in vivo competition experiments, since thus far this phenomenon cannot be studied in an in vitro system. Because these experiments require transient expression of deletion mutants of hMT-II$_A$ in primate cells and our original characterization of the hMT-II$_A$ regulatory region was based on stably transformed rat fibroblast lines, we wished to redefine some of the nucleotide sequence requirements for hMT-II$_A$ expression under conditions more similar to those of the competition assay. Deletion mutants of the hMT-II$_A$ 5′ flanking region (*6*) were fused to the bacterial gene coding for chloramphenicol acetyltransferase (CAT; *20*) (Fig. 1A) and were transfected into HeLa and CV-1 cells. Deletion of hMT-II$_A$ 5′ flanking DNA from the 5′-most Hind III site to position −160 relative to the start of transcription had no effect on either the basal or induced level of CAT expression (Fig. 1B). The fusion genes containing at least this amount of 5′-flanking DNA were induced 7- to 10-fold by Cd^{2+}. The extent of induction in different experiments ranged between 3- to 12-fold due to fluctuations in the basal level; however, within a single experiment, the response was much less variable. A deletion to position −96 decreased the basal level of promoter activity by almost one order of magnitude, but the mutant was still responsive to Cd^{2+}. A further deletion to position −50 abolished expression altogether. No significant differences were observed between HeLa and CV-1 cells with respect to expression of the fusion genes except for the higher transfection efficiency of the latter cell line.

In stably transformed Rat 2 cells, a deletion to position −96 had no significant effect on the basal level of promoter activity

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