

# The Effects of Heat Shock on the Morphology and Protein Synthesis of the Epidermis of *Xenopus laevis* Larvae

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**Abstract.** By scanning electron microscopy, we have observed that a 20-min heat shock at 37°C, although not lethal, causes extensive damage to the epidermis of 30-h and 2-d (post-fertilization) *Xenopus laevis* larvae. The primary effects of heat shock are the apical swelling of the epidermal cells, giving the epidermis a "cobblestone" appearance, and the selective shedding of the ciliated cells. The shed cells may be cell fragments, however, because some of them are anucleate. Shed cells also exhibit the enriched synthesis of a group of heat shock proteins of 62,000 D molecular

weight, suggesting that these proteins are specific to the shed cells. Prolonged heat shock of these larvae (i.e., 30 min at 37°C) results in the complete disintegration of the epidermis, followed by larval death. At later stages of development (3-d and 4-d post-fertilization), the epidermis becomes more resistant to heat-induced damage inflicted by a 20-min heat shock. This increase in resistance coincides with the development of large secretory cells and the loss of ciliated cells in the epidermis and thus parallels a change in the state of histological differentiation.

THE effect of heat shock on the physiology and metabolism of cells has been extensively studied. Most investigations have concentrated on analyzing the molecular responses of cells to thermal stress. During heat shock, cells selectively synthesize heat shock proteins (hsps),<sup>1</sup> which are believed to enhance their ability to survive at the higher temperature (for reviews see 2, 14, 19).

Heat shock can cause extensive cellular damage, particularly to membranous structures. These effects have been examined at the ultrastructural level and include the dispersal of the Golgi apparatus (23) and endoplasmic reticulum (3) and the swelling of mitochondria (23), which apparently lose the ability to function properly (12; for review see 11). The plasma membrane is also affected by heat shock, exhibiting increases in fluidity (13), loss of active transport activity (4) and formation of lesions large enough to allow the passage of small molecules (for review see 19). Nonmembranous structures also suffer heat-induced damage. The chromatin in nuclei condenses (20), whereas nucleoli disperse (13, 24). Bundles of actin filaments also form within the nuclei (20, 23), and in the cytoplasm, the intermediate filament network collapses onto the nucleus (23).

The effects of heat shock on intact organisms or tissues have been less well characterized. Embryos are highly susceptible to heat shock, however, as they often develop severe abnormalities if exposed to elevated temperatures during critical periods of their development. These effects are exemplified by mammalian embryos, which are particularly susceptible to heat-induced defects of the central nervous system

(22), and by *Drosophila* larvae, which can be induced by heat shock to develop phenocopies (15). How heat shock effects developing embryos is uncertain, although some lines of evidence suggest that elevated temperatures interrupt predetermined patterns of cell division in the case of mammalian embryos (for review see 8) or, alternatively, interfere with the expression of genes presumably required for normal differentiation in the case of *Drosophila* larvae (21).

Heat shock during critical periods of *Xenopus* development also disrupts the normal patterns of morphogenesis. If heat-shocked for 20 min at 35°C at the early blastula stage, *Xenopus* embryos lose the ability to gastrulate and instead the cells form three distinct clusters. The effect of heat shock is reduced if the embryos are treated as mid-blastulae. These embryos begin to gastrulate, but midway through the process, the endoderm flows out of the embryo toward the dorsal mid-line (exogastrulation). Interestingly, the mesodermal and ectodermal structures appear normal in these exogastrulae, suggesting that only endodermal derivatives of embryos at this stage are susceptible to heat shock. Heat-shocking the embryos after the mid-blastula stage has no gross effect on their development or survivability (17). Post-blastula embryos still experience minor abnormalities, however, after being heat-shocked at higher temperatures. Short heat shocks at 37°C disturb the normal patterning of somite formation, particularly in paraxial mesoderm that was in the process of differentiation at the time of heat shock (7).

We have investigated the effects of a 37°C heat shock on *Xenopus* larvae using the scanning electron microscope. Although larvae survive a short heat shock at 37°C, this treatment still causes extensive damage to the epidermis. Here,

1. Abbreviation used in this paper: hsp, heat shock protein.

we report that a 20-min heat shock at 37°C results in partial shedding of the epidermis, which can lead to the complete disintegration of this epithelium during an extended, and normally lethal, exposure to this temperature. This effect is only observed in early larvae, however, with the later stages becoming progressively more resistant to heat-induced damage.

## Materials and Methods

### Handling of Animals and Gametes

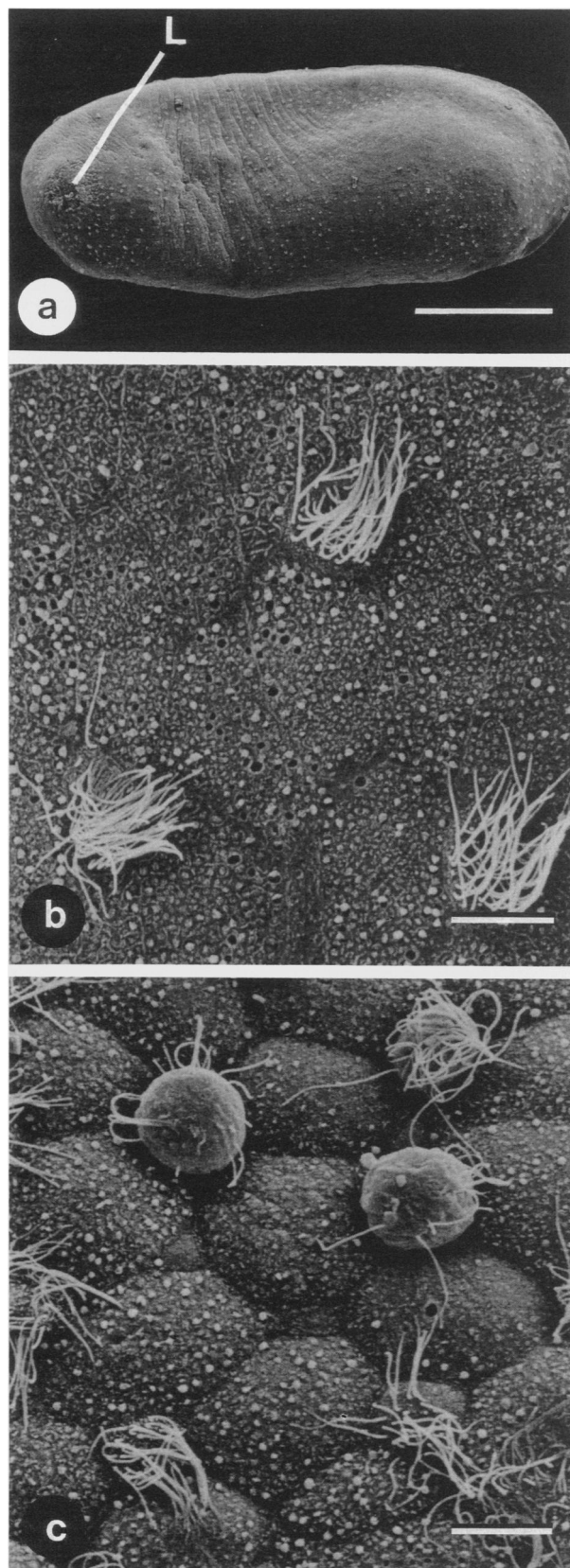
Sexually mature *Xenopus laevis* were purchased from Nasco Inc. (Ft. Atkinson, WI) and kept in tanks with continuously flowing dechlorinated water. To obtain gametes, we primed the frogs 10–12 h before spawning with a subcutaneous injection of chorionic gonadotropin from human pregnancy urine (Sigma Chemical Co., St. Louis, MO) in 0.65% (wt/vol) NaCl (females: 800 IU; males: 500 IU). The testes were dissected from males, killed in 0.5% wt/vol Tricaine (Sigma Chemical Co.) and stored at 4°C by the method of Newport and Kirschner (16) or used immediately for the artificial fertilization of eggs. For fertilizations, we macerated a single, or a half, testis in a small volume of 100% Modified Barth Solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO<sub>3</sub>, 0.82 mM MgSO<sub>4</sub>, 0.33 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.41 mM CaCl<sub>2</sub> and 10 mM Hepes buffer, pH 7.6), which was then pipetted into a dish containing eggs spawned into 34% Modified Barth Solution. Fertilization of the eggs was facilitated by gently rotating this mixture on a shaker for 5–10 min, after which the eggs were diluted with a large volume of Steinberg's solution (60 mM NaCl, 0.7 mM KCl, 0.8 mM MgSO<sub>4</sub>, 0.3 mM Ca(NO<sub>3</sub>)<sub>2</sub> and 1.4 mM Tris, pH 7.4). The embryos were allowed to develop in Steinberg's solution at a concentration of ~100 embryos per 30 ml of medium. During the course of development, abnormal embryos were removed, and the Steinberg's solution was changed every 10–12 h. In some experiments, the embryos were grown in filter-sterilized Steinberg's solution containing 100 U penicillin and 20 mg streptomycin/ml or 50 µg Gentamicin/ml.

### Heat-shocking and Fixation of Larvae

For this study, we used hatched larvae. This minimized handling and avoided steps such as chemical dejellying. For early larvae, we occasionally had to remove the jelly coat and fertilization envelope manually. Any larvae damaged by this procedure were discarded. At the appropriate stages of development, five larvae were placed in a 1.5-ml microfuge tube containing 100 µl of fresh Steinberg's solution. This tube was then submerged in a 37°C water bath for 20 min. Controls were treated in exactly the same way, but were kept at 22°C. At the end of the heat-shock period, the Steinberg's solution was removed and replaced with 1 ml of 3% glutaraldehyde in Millonig's phosphate buffer (123 mM NaH<sub>2</sub>PO<sub>4</sub>, 9.7 mM NaOH, pH 7.3) containing 2.7 mM glucose and 0.034 mM CaCl<sub>2</sub> for 30 min at 22°C. After fixation, the samples were washed twice with Millonig's buffer and stored in buffer at 4°C until all stages were ready for dehydration. This storage period did not affect the morphology of the samples. The samples were dehydrated in a graded series of ethanol. The samples were then critical-point dried in liquid CO<sub>2</sub> in a Bomar SPC-900/EX critical point drying apparatus and mounted on stubs with double-sided cellophane tape. Specimens were coated with a 25 nm gold–palladium film and viewed in a JEOL JSM-35CF scanning electron microscope operated at 10 kV.

### Labeling and Gel Electrophoresis

For the labeling of newly synthesized proteins, we heat-shocked larvae as described above, except that the Steinberg's solution contained 1 µCi per µl of [<sup>35</sup>S]methionine (Amersham, Oakville, Ontario, Canada; >800 Ci/mmol). After heat shock, the labeling medium was removed, and the larvae were homogenized in 25 µl of a buffer containing 120 mM Tris (pH 6.8), 10 mM EDTA and 0.1 mg/ml RNase A (Sigma Chemical Co.). This homogenate was incubated at 37°C for 5 min and then centrifuged at 13,000 rpm in a microfuge for 5 min. The cleared supernatant was combined with an equal volume of sample buffer (4% SDS, 20% β-mercaptoethanol, 0.2% glycerol and bromphenol blue) and boiled for 5 min. The incorporation of <sup>35</sup>S into proteins was determined by spotting 5 µl of each sample onto glass-fiber filters and washing them twice with ice-cold 25% TCA containing 1 mg/ml cold methionine, twice with ice-cold 8% TCA containing me-



**Figure 1.** Scanning electron micrographs of 30-h larvae. (a) Whole mount of a control larva. A rudimentary lens (*L*) is apparent in the head. (b) Flank epidermis of a control larva, showing principal cells and ciliated cells. (c) Flank epidermis of a heat-shocked larva. The principal cells have rounded apices, and some of the ciliated cells are elevated above the surface of the epidermis. Bars (a) 0.5 mm; (b and c) 10 µm.

thionine and twice with 95% ethanol cooled to  $-20^{\circ}\text{C}$ . The filter discs were air-dried and counted in a toluene:Spectrafluor (Amersham) cocktail in an LKB 1211 Rackbeta Counter.

For SDS-polyacrylamide gel electrophoresis, equal TCA-precipitable cpm of each sample were loaded into each well of a 4% polyacrylamide stacking gel buffered with 125 mM Tris (pH 6.8) containing 0.1% SDS, overlying a 10% polyacrylamide separating gel buffered with 375 mM Tris (pH 8.8) containing 0.1% SDS. Electrophoresis was carried out at  $15^{\circ}\text{C}$  at 25–30 mA constant current until the bromphenol blue tracking dye had reached the bottom of the gel. The gels were then stained for 15 min in 45% vol/vol methanol and 9% vol/vol acetic acid containing 0.25% wt/vol Coomassie Brilliant Blue R, followed by destaining overnight in 7.5% vol/vol methanol and 5% vol/vol acetic acid. For fluorography, destained gels were soaked in EnLightning (New England Nuclear, Dorval, Quebec), dried onto Whatman 3M paper and exposed to preflashed Kodak XAR5 film at  $-70^{\circ}\text{C}$ .

For some experiments, we labeled the cells shed from control and heat-shocked larvae. To isolate shed cells, we combined 10–15 larvae in a microfuge tube containing 100  $\mu\text{l}$  of Steinberg's solution. These larvae then were heat-shocked at  $37^{\circ}\text{C}$  for 11 min, and the medium was aspirated and placed into another tube. This tube was briefly centrifuged in a microfuge (10–15 s), and excess medium was aspirated to leave 10  $\mu\text{l}$ . Cells shed from control larvae were isolated in the same way, except that they were not given an 11-min heat shock. For labeling, we added 30–45  $\mu\text{Ci}$  of [ $^{35}\text{S}$ ]methionine to the cells and continued heat-shocking them at  $37^{\circ}\text{C}$  for another 20 min. Control cells were left at ambient temperature. After heat shock, we added 12  $\mu\text{l}$  of the Tris/EDTA/RNase buffer containing 0.5% SDS to the tubes, homogenized the cells and incubated them at  $22^{\circ}\text{C}$  for 5 min. This was followed by the addition of 25  $\mu\text{l}$  of sample buffer and boiling for 5 min. The samples were then counted and were subjected to electrophoresis as described above.

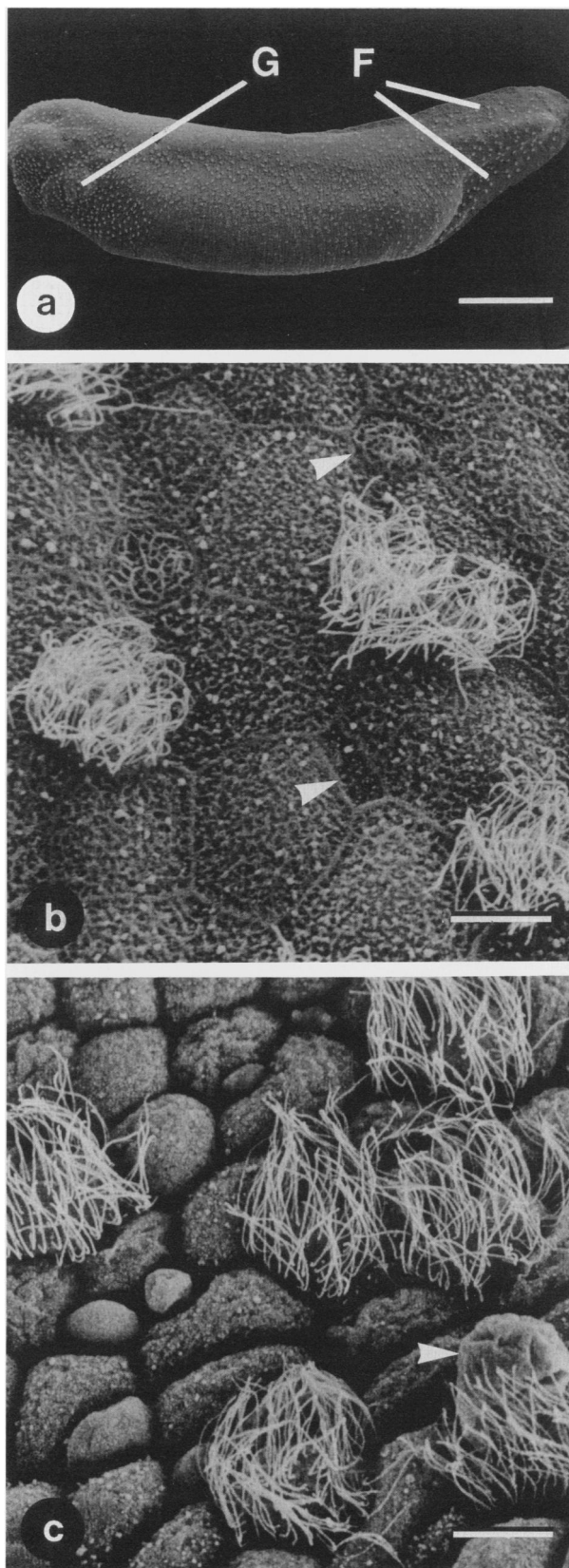
### Light Microscopy

Cells shed by larvae were isolated as described above and observed using an Olympus CK inverted phase-contrast microscope or photographed using an Olympus BH-2 compound microscope with phase-contrast optics. For Hoechst 33342 staining, we isolated shed cells and placed them in a 100- $\mu\text{l}$  droplet of medium on a plastic slide, added 1  $\mu\text{l}$  of a 2 mM concentrated stain solution to the droplet and incubated the cells in the dark for 1 h. The medium was then aspirated, and the cells were gently squashed with a coverslip. Fluorescence of the cells was stimulated by ultraviolet light (360 nm).

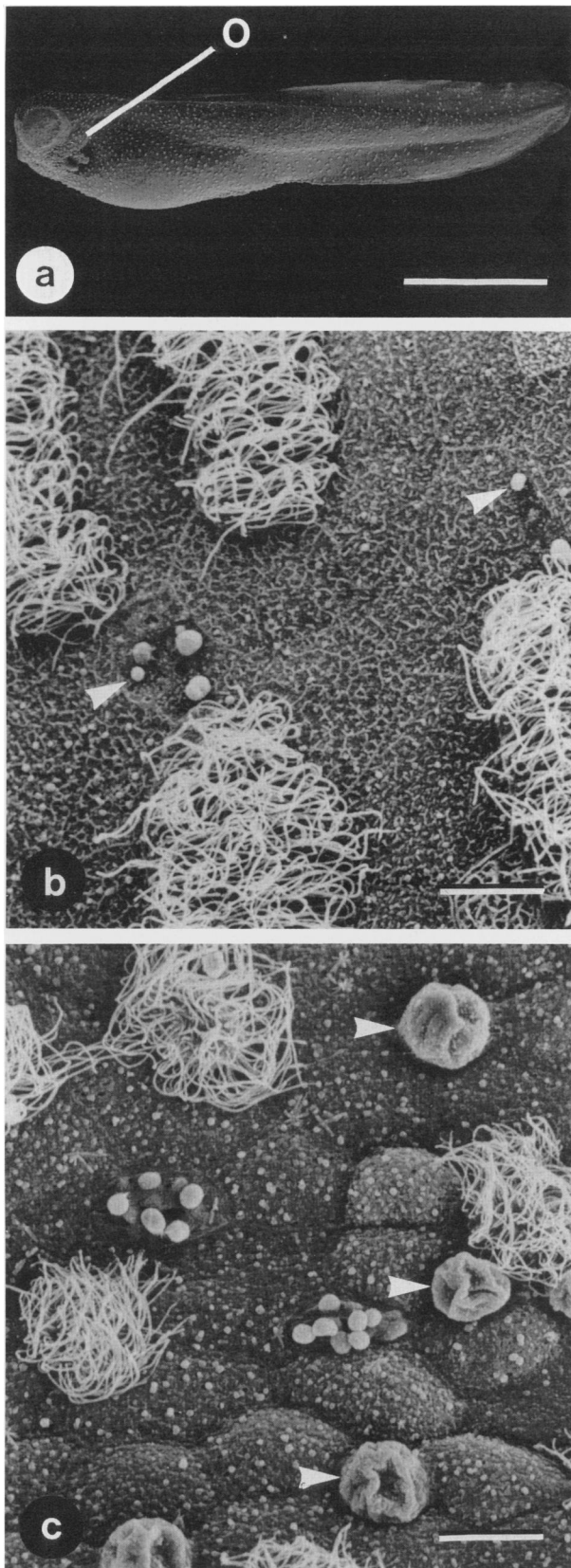
## Results

### Heat Shock-induced Effects on the Epidermis of Different Stages of Larvae

We have examined the effects of heat shock on the integrity of the epidermis of *Xenopus laevis* larvae at four stages of development. These stages are shown in the photomicrographs in Figs. 1–4 and include larvae at 30 h (Fig. 1a) and  $\sim 2$  d (Fig. 2a), 3 d (Fig. 3a) and 4 d (Fig. 4a) post-fertilization. These stages correspond to the Nieuwkoop and Faber stages 24–25, 34–36, 41–42, and 45, respectively (18). Surface details of these larvae grown at ambient, “control” temperature ( $22^{\circ}\text{C}$ ) or after a 20-min heat shock at  $37^{\circ}\text{C}$  were visualized by scanning electron microscopy. A 20-min heat shock causes severe damage to the epidermis of both the 30-h and 2-d larvae. The epidermis of these larvae is composed of at least two distinct cell types. The principal cells are polygonal in shape with a flat apical surface that shows evidence of exocytotic activity. The epidermis is punctuated with single cells bearing 200–300 cilia on their apical surface (Fig. 1b and 2b). Small secretory cells are also detected in the epidermis of the 2-d larvae (Fig. 2b). Distinct boundaries are evident between the cells. After heat shock, however, the epidermis from both larval stages is rough, assuming a “cobblestone” appearance (Figs. 1c and 2c). This effect might be caused by the expansion of the apical surface of the principal cells,



**Figure 2.** Scanning electron micrographs of 2-d larvae. (a) Whole mount of a control larva. External gills (G) and dorsal and ventral fins (F) are developing. (b) Flank epidermis of a control larva, revealing the appearance of putative small secretory cells (arrowheads) between the principal cells and the ciliated cells. (c) Flank epidermis of a heat-shocked larva. A large apical bleb is evident on one cell (arrowhead) in this field. Deep clefts ring the apices of all epidermal cells. Bars (a) 0.5 mm; (b and c) 10  $\mu\text{m}$ .



**Figure 3.** Scanning electron micrographs of 3-d larvae. (a) Whole mount of a control larva. An opercular fold (*O*) adjoins the external gills. (b) Flank epidermis of a control larva, illustrating principal cells, ciliated cells, and large secretory cells. Mucigen droplets (*arrowheads*) adhere to the apices of the secretory cells. (c) Flank

which still appear to be firmly anchored to each other. Many of the ciliated cells are elevated above the surface of the epidermis, however, and are in the process of being shed (see below).

As the larvae develop, the epidermis becomes less affected by heat shock. This reduction in heat-induced damage parallels a change in the specialized cell types in the epidermis. By 3 d of development, larvae are beginning to reduce the number of ciliated cells, apparently by ciliary resorption, and increase the number of large secretory cells (Fig. 3 *b*), which become very prominent in the epidermis of the 4-d larvae (Fig. 4 *b*). These secretory cells may be derived from the small secretory cells evident in the epidermis of 2-d larvae. In some larvae, the fixation procedure was able to preserve large droplets of mucigen from the discharging vacuoles of these cells (Figs. 3, *b* and *c*, and 4 *c*), whereas in others, these droplets are absent (Fig. 4 *b*).

The effects of heat shock on 3-d and 4-d larvae are illustrated in Figs. 3 *c* and 4 *c*, respectively. Heat shock of 3-d larvae results in a less pronounced "cobblestone" appearance, although some cells form a large bleb at their apical surface. Occasionally, similar blebs were also observed in the epidermis of heat-shocked 2-d larvae (see Fig. 2 *c*). The ciliated cells appear to be unaffected by heat shock (Fig. 3 *c*). The epidermis of 4-d larvae is even less affected by heat shock. These cells do not acquire the "cobblestone" appearance typical of the other stages of development (Fig. 4 *c*).

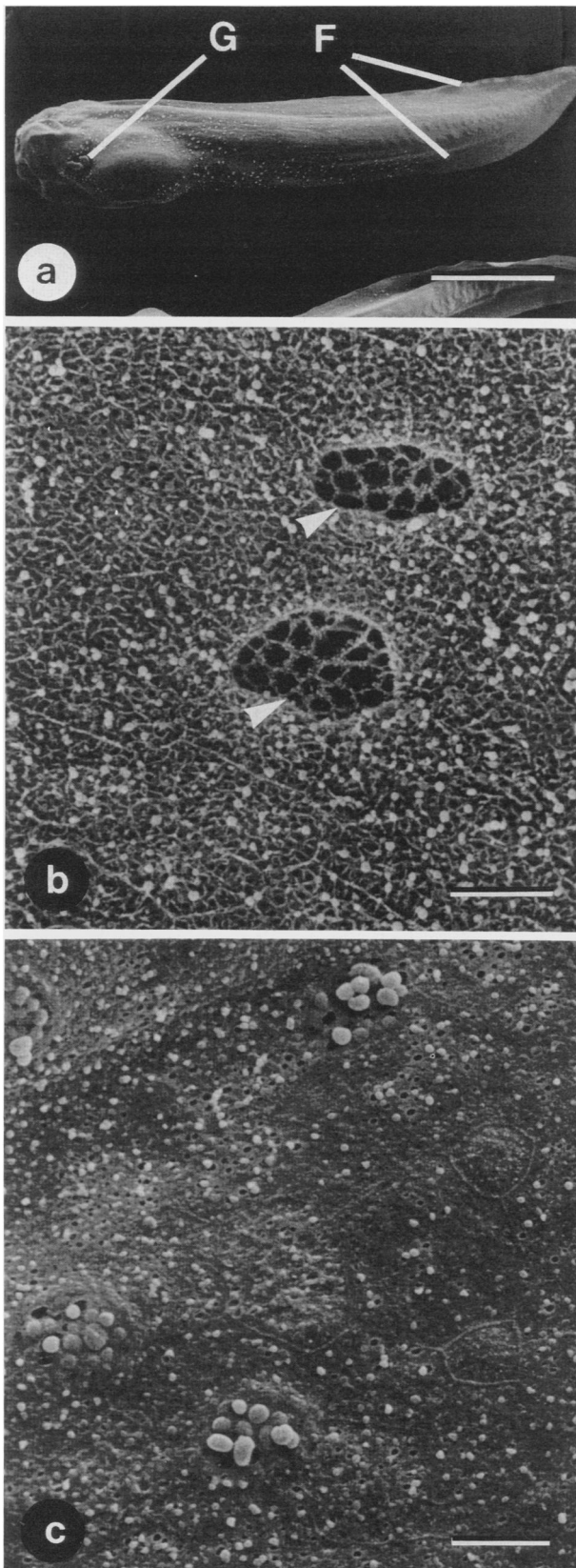
#### ***Heat-shocked 30-h and 2-d Larvae Shed Their Epithelial Cells***

We have observed that 30-h and 2-d larvae shed cells during heat shock. This shedding process begins with the selective loss of the ciliated cells and eventually leads to disintegration of the epidermis (see below). The selective loss of ciliated cells is documented in Fig. 5, a series of scanning electron micrographs showing ciliated cells in the process of leaving the surfaces of 30-h and 2-d heat-shocked larvae (the process of shedding is indistinguishable between the two stages of development). Fig. 5 *a* shows a ciliated cell of a control larva for comparison. During heat shock, some of these cells appear to elevate from the surrounding cells (Fig. 5 *b*). These elevated cells are sometimes broken open, presumably as an artefact of sample preparation (Fig. 5 *c*). This particular cell has left behind a substantial amount of cytoplasm, implying that its basal surface is tightly anchored to neighboring cells and/or the underlying matrix. The elevated cells eventually become free from the epidermis, however, and are often observed upside-down or lying on their side (Fig. 5 *d*, see also Fig. 1 *c*). In addition, we are able to detect numerous ciliated cells in the medium in which the larvae are heat-shocked (Fig. 6 *a*).

The preferential shedding of ciliated cells is documented in Table I. For this experiment, ten 2-d larvae were placed in 100  $\mu$ l of Steinberg's solution and heat-shocked at 37°C for 20 min or left at 22°C. The medium was then removed and placed on a glass coverslip, which was left undisturbed to al-

epidermis of a heat-shocked larva. Only some of the principal cells have rounded apices. Large apical blebs (*arrowheads*) characterize several cells in this field. Bars: (a) 1 mm; (b and c) 10  $\mu$ m.





**Figure 4.** Scanning electron micrographs of 4-d larvae. (a) Whole mount of a control larva. F, fin; G, external gill. (b) Flank epidermis of a control larva. Mucigen droplets were not preserved, exposing the exocytotic vacuoles opening onto the apical surfaces of the secretory cells (arrowheads). (c) Flank epidermis of a heat-shocked

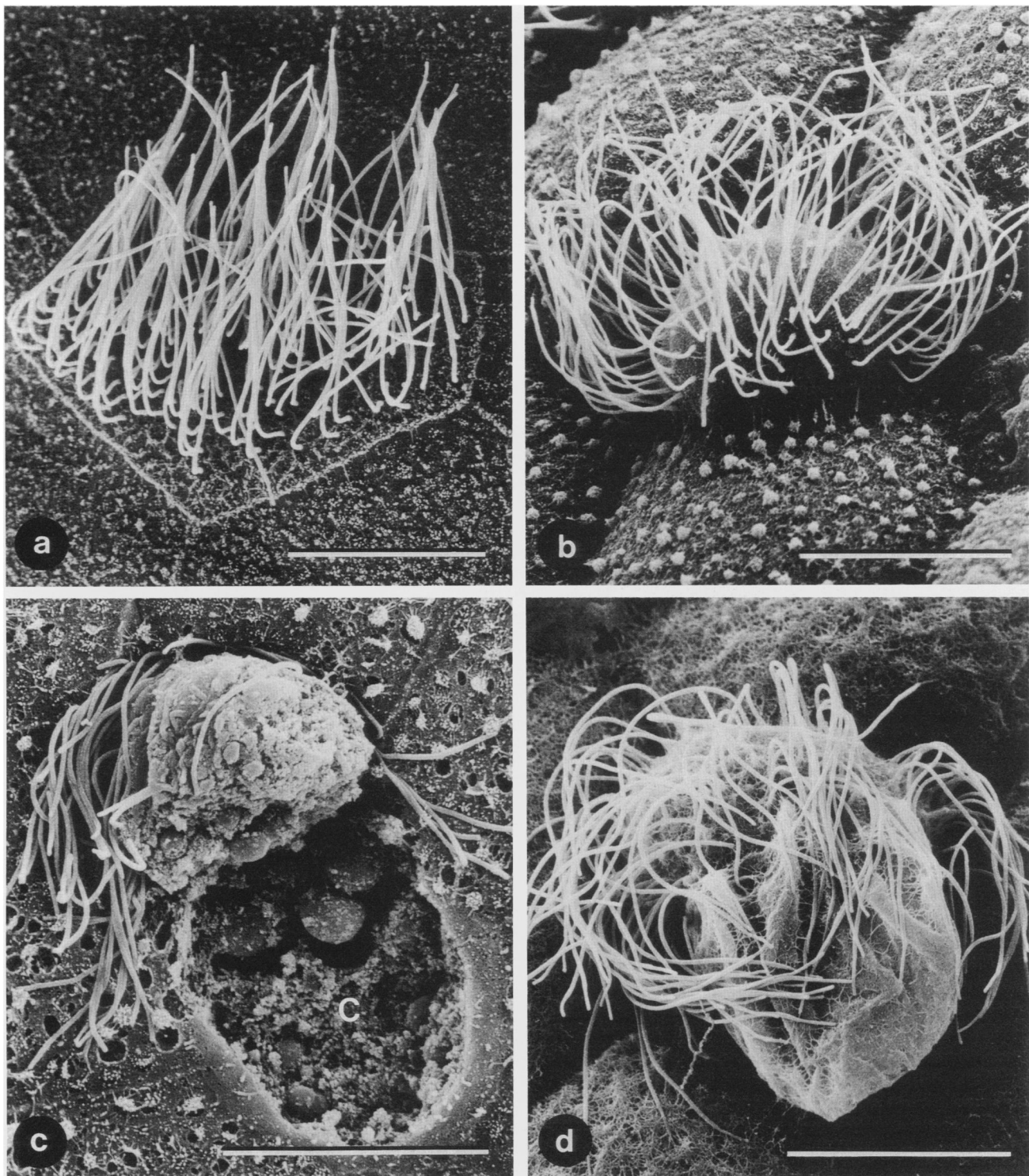
low the cells to settle. The settled cells were observed through an inverted phase-contrast microscope, allowing us to count the number of cells present and score them for cilia. It is apparent that heat-shocked larvae shed significantly more cells than do their control counterparts. Approximately 62% of the cells shed during heat shock are ciliated, whereas only 14% of the cells in the intact epidermis are ciliated. The shedding of ciliated cells into the medium also corresponds to a significant decrease in the percentage of ciliated cells in the epidermis of heat-shocked larvae as compared with controls.

It is relevant to note that the shedding of ciliated cells includes a process of cell fragmentation. This is demonstrated by the fact that we were only rarely able to identify gaps in the epidermis of heat-shocked larvae. Instead, areas presumably once occupied by ciliated cells appear to contain a small cellular "plug" (data not shown). Also, some of the shed cells do not contain nuclei. This is evident in Fig. 6, b and c, which show two shed ciliated cells exposed to Hoechst stain. The presence of a nucleus is detected in only one of the two cells in the field. The apparent lack of nuclei in some shed cells suggests that they are apical cytoplasmic fragments and that their nuclei were retained by basal fragments left behind in the epidermis. In general, all the cells exhibit low level fluorescence, possibly due to the staining of mitochondrial DNA.

#### ***Shed Cells Synthesize a Modified Pattern of Heat-Shock Proteins***

Shed cells are metabolically active as demonstrated by their ability to synthesize hsp's. *Xenopus* embryos synthesize four major hsp's of 87-, 76-, 70-, and 62- (formerly 57-) kD molecular masses (9, 17), with the additional synthesis of several minor hsp's depending on the conditions used to heat shock the embryos (Nickells, R. W., L. W. Browder, and T. I. Wang, manuscript in preparation). Fig. 7 a shows the radioactively labeled proteins synthesized by 2-d larvae under control and heat shock conditions. Heat-shocked larvae synthesize the four major hsp's. When proteins are extracted from an aliquot of medium containing shed cells and analyzed by SDS-gel electrophoresis, however, we detect only a subset of hsp's (Fig. 7 b). Consistently, the cells in the medium contain only hsp's 76 and 70 and a complex of hsp's at 62 kD. The synthesis of hsp70 is greatly reduced, however. This modified pattern of hsp synthesis appears to be a characteristic of shed cells. Shed cells were isolated and then labeled with [<sup>35</sup>S]methionine (Fig. 7 c). These cells show detectable synthesis of hsp's 76 and 70 and the 62 complex. As in the medium samples, the synthesis of hsp70 is greatly reduced. We have investigated the possibility that this pattern of hsp synthesis is characteristic of all cells in the epidermis. However, the cells of isolated epidermis strongly synthesize hsp70, as well as hsp's 76 and 62. A similar pattern of hsp's is also detected in the underlying axial tissues, although the synthesis of hsp62 is somewhat reduced (data not shown).

larva. Except for the preservation of mucigen droplets, this epidermis is indistinguishable from the control epidermis. Bars (a) 1 mm; (b and c) 10  $\mu$ m.

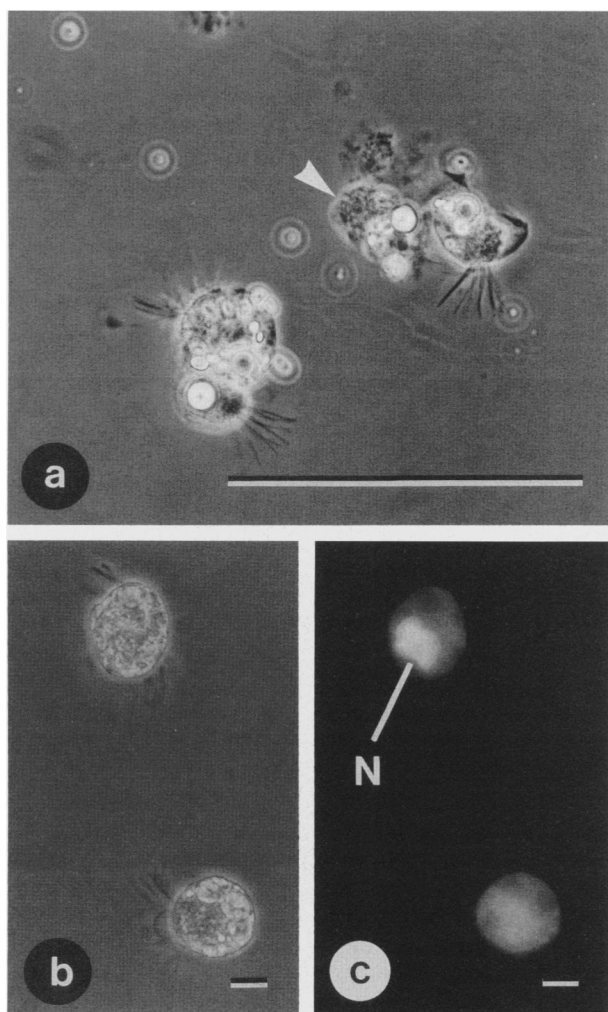


**Figure 5.** Scanning electron micrographs of ciliated cells in the larval epidermis. (a) Typical ciliated cell of a control larva (2-d). Note the flattened cellular apex and the boundary with the adjoining principal cells. (b) Elevating ciliated cell of a heat-shocked larva (30-h). (c) Elevating ciliated cell of a 30-h larvae that has broken open to reveal its cytoplasm (C). (d) Shed ciliated cell on the surface of a heat-shocked larva (2-d). Bars, 10  $\mu$ m.

### ***Prolonged Heat Shock Causes the Disintegration of the Larval Epidermis***

As described above, a 20-min heat shock at 37°C causes the preferential shedding of ciliated cells from the epidermis of 30-h and 2-d larvae. This phenomenon precedes a much

more extensive breakdown of the epidermis, which can often be observed in the posterior regions of 2-d larvae heat-shocked for 20 min (Fig. 8 a). It is apparent that all cells are losing their intercellular attachments and breaking free. An overall weakening of the attachment of the epidermis to the



**Figure 6.** Photomicrographs of epidermal cells shed into the medium by a heat-shocked 2-d larva. (a) Phase-contrast image of four shed cells. One epidermal cell (arrowhead) in this group is not ciliated. (b) Phase-contrast image of two shed cells stained with Hoechst dye. Both epidermal cells are ciliated. (c) Fluorescence image of the same cells shown in b. Excitation of the Hoechst dye with ultraviolet light reveals that one cell possesses a nucleus (N) and the other does not. Bars (a) 100  $\mu\text{m}$ ; (b and c) 10  $\mu\text{m}$ .

underlying tissues may also contribute to this phenomenon. This weakening is evident in heat-shocked larvae that have been dried for scanning electron microscopy, because large pieces of epidermis often fall off the specimens while they are being mounted on stubs (Fig. 8 b). A weakening of the epidermal attachment is also apparent in 2-d larvae that have been given a longer heat shock (30 min at 37°C). This slightly longer heat treatment results in an even greater loss of epidermal cells as detected by light microscopy of free cells in the medium. Under these heat-shock conditions, the majority of cells observed in the medium are non-ciliated, occurring either free or in large epidermal flakes (Fig. 8 c). Unlike the short heat shocks, from which all the stages of larvae examined can fully recover and develop normally, these longer heat shocks are lethal to 2-d larvae, which continue to develop for a short time and then literally fall apart (data not shown).

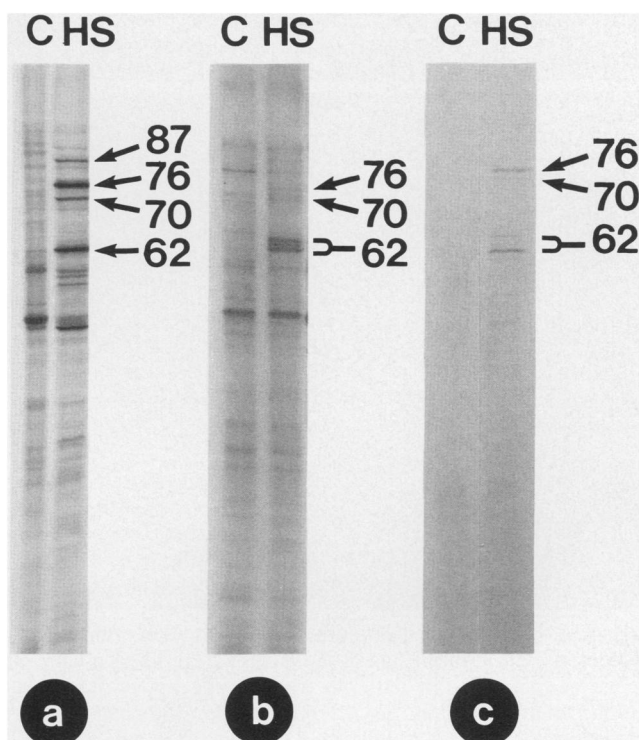
**Table I. Percentages of Ciliated Cells Shed into the Medium versus in the Intact Epidermis**

Location	Total number of cells	Total ciliated	Percentage
<b>Medium*</b>			
Control	5	0	0
Heat shock <sup>‡</sup>	138	86	62
<b>Epidermis<sup>‡</sup></b>			
Control	182	27	14.8
Heat shock <sup>‡</sup>	192	13	6.8

\* The numbers represent the total number of cells observed based on three separate counts of the cells that had settled on a glass coverslip.

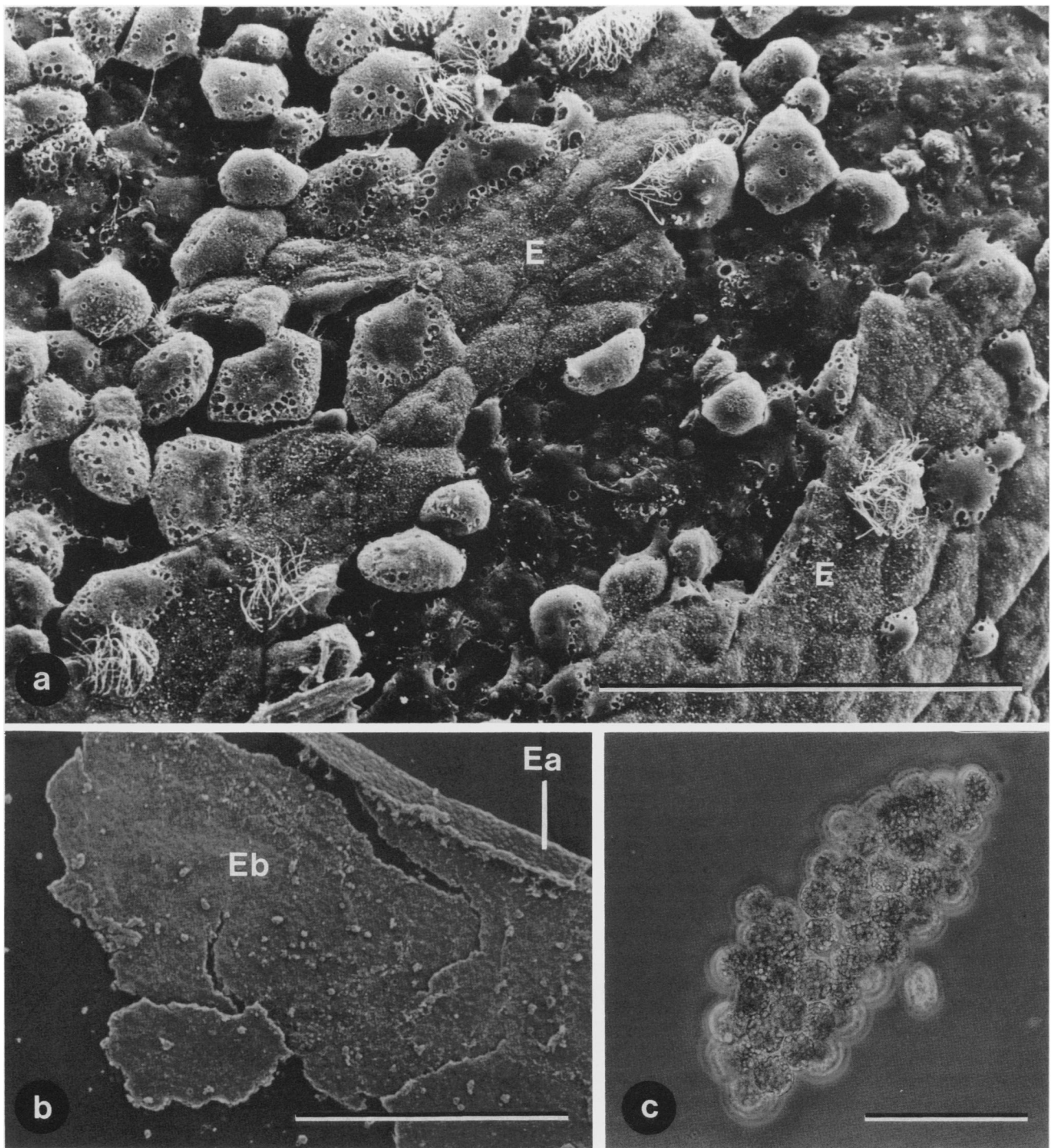
‡ The number of cells in the epidermis was determined by counting cells from photographs taken of the flanks of larvae using the scanning electron microscope. The numbers shown represent the totals from four views of two larvae (controls) and two views of one larva (heat shock).

§ The ratios of ciliated cells in the heat-shocked samples were tested against the ratio of ciliated cells found in the epidermis of control larvae for significant differences by  $\chi^2$  analysis. There is a significantly higher ratio of ciliated cells in the medium of heat-shocked larvae than would be expected by random loss of cells ( $p < 0.001$ ) and a significantly lower ratio of ciliated cells in the epidermis of heat-shocked larvae ( $0.001 < p < 0.005$ ). Both analyses confirm that ciliated cells are preferentially leaving the epidermis of heat-shocked larvae.



**Figure 7.** Fluorographs of [<sup>35</sup>S]methionine labeled proteins of 2-d larvae separated on SDS-10% polyacrylamide gels. (a) Whole larvae. (b) Aliquots of the labeling media that contain shed cells. (c) Shed epidermal cells that have been isolated and then labeled for 20 min at 22°C or 37°C. Control (C) and heat shock (HS) lanes are shown for each sample. The apparent molecular masses of the heat shock proteins are given in kD. In b and c, the HS lanes show a complex of hsp's of approximately 62-kD molecular mass. The actual molecular masses of these proteins are 65, 62, and 61 kD.





**Figure 8.** Micrographs illustrating the disintegration of the epidermis of heat-shocked 2-d larvae. (a) Scanning electron micrograph of the epidermis (E) in the region of the posterior tail fin of a larva heat-shocked for 20 min at 37°C. (b) Scanning electron micrograph of a piece of epidermis that fell away from a critical-point dried, heat-shocked larva while it was being mounted for observation. The basal surface of the epidermis (Eb) is exposed to view, although a fold in the specimen reveals part of the apical surface (Ea) with its "cobblestone" appearance. (c) Phase-contrast photomicrograph of a flake of epidermis shed into the medium by a larva that was heat-shocked for 30 min at 37°C. Bars (a and c) 100 µm; (b) 500 µm.

## Discussion

We have documented the effects of heat shock on the integrity of the epidermis of *Xenopus* larvae by scanning electron microscopy. A 20-min heat shock at 37°C of early larvae causes

the selective shedding of ciliated cells, which is followed by complete epidermal disintegration if the larvae are exposed to further heat shock. The effects of a 20-min heat shock are less severe to older larvae, however, as they become progressively more resistant to this treatment. This enhanced resis-



tance is concurrent with a change in the cell specialization of the epidermis involving the normal development of large secretory cells and a decrease in the number of ciliated cells.

### **The Developmental Changes of *Xenopus* Larval Epidermis**

The normal morphology of the *Xenopus* epidermis is essentially the same as that described for other Anurans (*Rana pipiens*—10; *Bombina orientalis*—6). Early larval epidermis is composed of at least two cell types: those with cilia and those without. Both *Rana* (10) and *Xenopus* larvae also exhibit small, specialized secretory cells in addition to the general secretory activity of the principal cells. Ciliated cells persist through hatching, after which the cilia are resorbed in both species (10, our unpublished data). In *Xenopus*, as in *Rana*, the resorption of cilia coincides with the formation of large secretory cells and an increase in larval motility (i.e., they become free swimming). In *Rana*, this in turn correlates with the internalization of gills, suggesting that the function of cilia is to aid the larvae in respiration by moving water over the external gills (10).

### **Heat Shock-induced Changes in Epidermal Morphology**

A 20-min heat shock at 37°C produces dramatic changes in the epidermis of 30-h and 2-d larvae, giving the normally flat layer of cells a “cobblestone” appearance and causing the selective shedding of the ciliated cells. These phenomena are probably not a result of changes in intercellular attachments, since the cells appear to still be firmly anchored to each other. Instead, the effect seems to be due to the expansion of the apical surface of the cells, possibly as a result of an inability to regulate their osmotic potential during heat shock, which causes them to swell with water. A similar effect has been observed in the cells of heat-shocked *Drosophila* salivary glands. These cells increase their volume by ~16%, which the authors suggest is due to an uptake of water associated with changes in the ion concentrations within the cells (5).

Apical swelling may also result in the fragmentation of the cells. As they expand, the apical regions would eventually break free near the level of their attachments to neighboring cells. This method of fragmentation would also favor the selective loss of ciliated cells, which are more active than nonciliated cells and would be more likely to break themselves free. During the course of the experiments reported here, we were able to observe the epidermis in the thin tail regions of whole 2-d larvae in petri dishes using an inverted phase-contrast microscope. After heat shock, the ciliated cells were observed to continue beating their cilia, but often they were elevated above the epidermis and appeared to be spinning (our unpublished observations). These observations indicate that the action of the cilia may contribute to the cellular shedding.

In the more delicate areas of the larvae, such as the posterior tail fin, a 20-min heat shock can also cause disintegration of the epidermis. This phenomenon may be a result of the degeneration of the intercellular attachments, as suggested in Fig. 8 *a*, which shows cells on the periphery of a sheet of epidermis breaking free. Heat shock may also cause the weakening of the basal attachments of the epidermis as

suggested by the fragility of the skin of heat-shocked, critical-point dried larvae (Fig. 8 *b*). Both these effects appear to be accentuated by longer heat shocks, which result in the complete disintegration of the epidermis and eventual larval death.

The effects of a 20-min heat shock are particularly revealing because later stages of larval development are apparently unaffected by this treatment. This resistance may reflect any number of changes associated with the development of the epidermis, such as increases in the number of cell layers, changes in the type or number of intercellular junctions, or changes in the underlying extracellular lamina. We are continuing to investigate the responses of larval epidermis to heat shock in thin sections and freeze-fracture replicas. These techniques should help to resolve some of the questions stimulated by this scanning electron microscopic study.

### **Epidermal-specific Synthesis of Hsps**

We have observed that shed cells favor the synthesis of hsp76 and a complex of hsps of ~62 kD. Hsp76 and a single hsp from the 62-kD complex are also observed in the hsp synthetic profile of whole larvae. Occasionally, we also detect other members of the hsp62 complex in whole neurulae and larvae, but this complex is consistently observed in heat-shocked shed cells. This selective hsp synthesis may be stimulated by the process of shedding. Alternatively, the hsp62 complex may be selectively synthesized by ciliated cells, which are enriched in the population of shed cells, making the detection of these hsps more likely. This latter possibility may be difficult to examine, because, although enriched in ciliated cells, shed cells still represent a heterogeneous population of epidermal cells.

This study has shown that the epidermis of early larvae is susceptible to heat shock-induced damage, although the treatment is not necessarily lethal to the organism. Studies of this kind may prove to be especially relevant in understanding the effects of heat shock on whole tissues, especially in light of advances in hyperthermic therapy now used in the treatment of some kinds of human cancers (for reviews see 1).

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