Int. J. Dev. Biol. 37: 459-466 (1993)

Original Article

Mechanism of closure of experimental excision-wounds in the bare upper layer of the chick blastoderm

HILDE BORTIER[1]*, STEFAAN VANDEVELDE[2], LUCIEN C.A. VAKAET[3]

[1]*Department of Anatomy, Embryology, Histology, University of Gent,* [2]*Department of Dentistry and* [3]*Laboratory for Experimental Cancerology, University Hospital, Gent, Belgium*

ABSTRACT The closure of experimental excision-wounds in the upper layer of the gastrulating chick blastoderm was studied by time-lapse videography and videomicrography and by light microscopy, transmission electron microscopy and scanning electron microscopy. One experimental excision-wound was made in the upper layer of stages 4V to 6V blastoderms (Vakaet, *Arch. Biol. (Liège) 81:* 387-426, 1970), in the proamnion where no middle layer cells are present. The deep layer was previously discarded, so that the wounds were made in the bare upper layer. They closed within 2 to 6 hours and further development was normal by *in vitro* standards. With videography, global movements of the upper layer towards the wound were observed. With videomicrography, the wound submarginal region cells were seen to move like sheep in a flock: individual cells in different directions, the whole flock towards the wound. During closure the shape of the wound edge was irregular. The structure of the epithelium of the wound submarginal region was unchanged throughout closure: a pseudostratified columnar epithelium in which cell divisions occur at its dorsal side and are parallel to its surface. The basal lamina was absent below the edge of the wounds. We propose that the cells of the upper layer are mobile against one another and are not confined to a specific part of the basal lamina. During wound closure the movements of the cells on the basal lamina would be driven by mitotic pressure. This is the horizontal pressure exerted by the addition of daughter cells and their parting during anaphase and telophase. The parting of the anaphases and telophase cells brings about changes in the relative position of their neighbor cells as is demonstrated by the presence of connecting cords. The individual movements of cells result in a global movement of the upper layer towards the wound where no mitotic pressure exists. The movements in the upper layer to close a wound and the movements in the Anlage Fields during gastrulation might be driven by the same mitotic pressure.

KEY WORDS: *wounds, closure, videomicrography, gastrulation, mechanism*

Introduction

Healing of adult mammalian skin proceeds by epidermal cell migration and contraction of granulation tissue (Gabbiani *et al.*, 1978). Mitotic activity is only enhanced after 3 to 5 days (Peacock, 1985).

Weiss and Matoltsy (1959) stated that wound healing in the chick embryo is only possible after 10 days of incubation, as only from that stage on are epithelial cells able to migrate. Stannisstreet *et al.* (1980) considered enhancement of mitotic activity as an important factor in wound healing of straight cuts in the upper layer of the chick blastoderm. Thevenet (1984) also imputed wound healing in the skin of the back of 3- to 7-day-old chick embryos to enhancement of mitotic activity. The role of enhancement of mitotic activity in embryonic wound healing is, however, not generally accepted.

Lash (1955) described in Urodeles that embryonic wounds heal by movements of the epithelial layer. Ihara *et al.* (1990) explained wound healing in the 16-day-old rat skin in culture by a centripetal movement of the surrounding skin. Martin and Lewis (1992) described how in the 4-day-old chick wing bud the epidermis moves over the mesenchyme cells. They suggest that an actin cable in the edge acts as a contractile purse string to close up the wound. Mareel and Vakaet (1977) used time-lapse cinemicrophotography to study wound healing of the chick deep layer after partial removal. They observed migration of the healing cells on the ventral side of the upper layer.

Abbreviations used in this paper: SEM, scanning electron microscopy; TEM, transmission electron microscopy

***Address for reprints**: University of Gent, Department of Anatomy, Embryology, Histology, Godshuizenlaan 4, B-9000 Gent, Belgium. FAX: 32.9.2259452.

0214-6282/93/$03.00

Printed in Spain

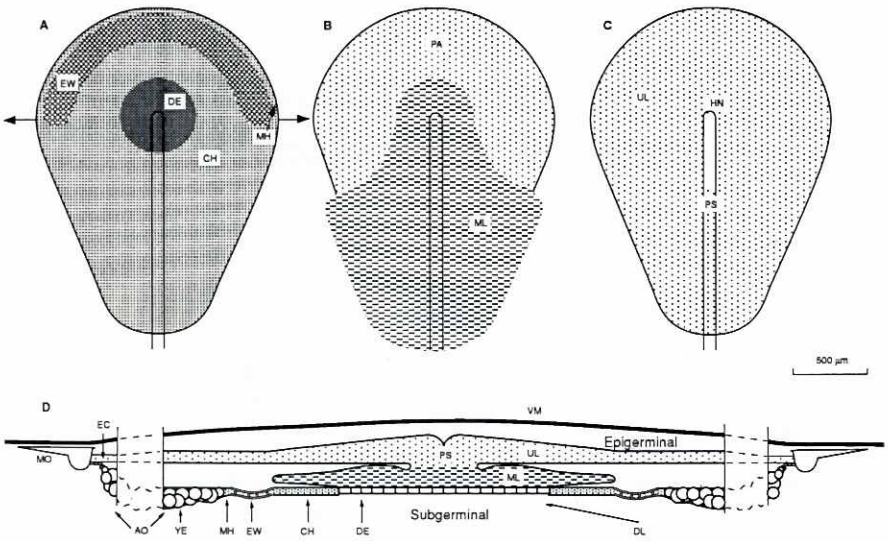

Fig. 1. Stage 5V chick blastoderm observed from the ventral side (A, B, C) and on a transverse section (D). (A) *Deep layer,* **(B)** *after resection of the deep layer,* **(C)** *upper layer after resection of the deep layer and the middle layer,* **(D)** *section in A (horizontal arrows). AO: area opaca, CH: central hypoblast, DE: definitive endoblast, EC: extraembryonal ectoblast, EW: endophyll wall, HN: Hensen 's node, MH: marginal hypoblast (arrow), ML: middle layer, MO: margin of overgrowth, PA: proamnion, PS: primitive streak, UL: upper layer, YE: yolk endoderm, VM: vitelline membrane.*

The former studies concern wound healing, since at least one other layer participated in the process. We studied wound closure in the bare upper layer of the chick blastoderm. During xenografting of Anlage Fields (Bortier and Vakaet, 1992a,b) we observed the readiness of the upper layer to heal. According to Gallera and Castro-Correia (1960) wounds in the upper layer of gastrulating chick blastoderms enlarge unless they are re-covered with deep layer. We observed that wounds in the upper layer could close without being re-covered with deep layer (Bortier, 1987; Bortier and Vakaet, 1987). This led us to study wound closure experimentally, documented with polaroid photography and time-lapse videography and videomicrography. Closure of the wounds was interrupted after various periods of time for study with light microscopy, TEM or SEM. Mitotic activity was studied quantitatively.

We propose a novel mechanism for the movements of cells in the upper layer during wound closure and for the morphogenetic movements of avian gastrulation.

Results

At stage 5V the deep layer of the chick blastoderm is composed of marginal hypoblast, endophyll, central hypoblast and definitive endoblast (Vakaet, 1970) (Fig. 1). After resection of the anterior part of the deep layer, one experimental excision-wound per blastoderm was made in the bare upper layer of the proamnion, where no middle layer cells are present (Fig. 2). Due to the translucency of the upper layer, wound closure could be followed with stereomicroscopy and more particularly with time-lapse videography and videomicrography.

Direct observation with stereomicroscopy (n= 403)

We observed 403 experimental excision-wounds in the bare upper layer. 48 wounds enlarged, 355 were closing or were closed at fixation. Wounds closed between 2 to 6 hours and further development of the blastoderms was normal by *in vitro* standards.

During the first seconds after wounding, fluid was squeezed through the wound from the epigerminal to the subgerminal space (Fig. 3A,B). The albumen-derived macromolecular material of the epigerminal space (Callebaut, 1982, 1983) had been brought under pressure by the stretching of the vitelline membrane against the culture medium. This stream, visible by the movement of yolk and debris away from the wound, stopped when sufficient epigerminal fluid had escaped to level the pressures in the epi- and subgerminal spaces.

The wounds showed a viscoelastic reaction upon wounding: they enlarged within one second, immediately followed by a narrowing, until wound diameters were between the original and largest size. After these reactions the wound submarginal region was thickened and curled ventrally.

Time-lapse videography (n= 38)

We followed 38 wounded blastoderms with time-lapse videography. Some minutes after reincubation the blastoderms flattened and epiboly by the margin of overgrowth resumed. After about 15 to 30 minutes yolk and debris moved towards the wound, revealing a fluid stream directed from the subgerminal to the

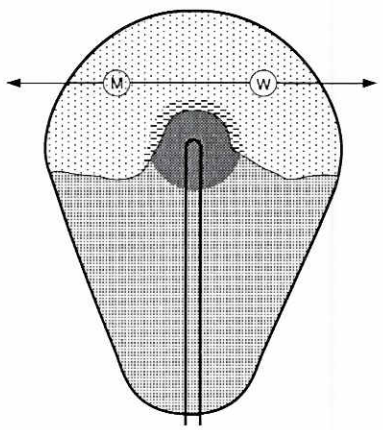

Fig. 2. Wound and mirror image area in a stage 5V blastoderm. *The deep layer is resected, wound (W) in the upper layer, mirror image area (M). Transverse arrowed line indicates the situation of the sections in Fig. 3.*

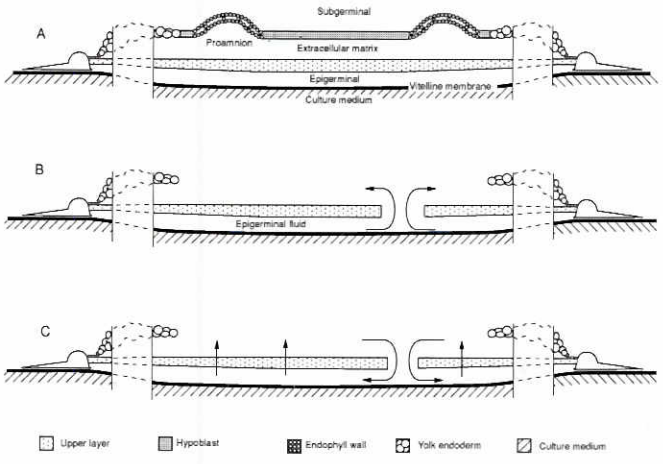

Fig. 3. Transverse sections through the proamnion region in a stage 5V chick blastoderm. *The blastoderm is stretched on the vitelline membrane.* **(A)** *Intact blastoderm.* **(B)** *Bare upper layer of the proamnion after wounding. Bent arrows: direction of the fluid stream from epigerminal to subgerminal.* **(C)** *Same wound as in B but during closure. Bent arrows: direction of the fluid stream from subgerminal to epigerminal. Straight arrows: direction of pumping of fluid through the upper layer.*

epigerminal space (Fig. 3C). It persisted as long as the wounds were open. It was generated by the physiological pumping of fluid through the upper layer, demonstrated by New (1955) and studied further by Jaffe and Stern (1979) and Stern *et al.* (1985).

The curled submarginal regions of the wounds unrolled like a carpet. By then, two parts of the wound periphery became distinct: the edge and the submarginal region. The edge cells at the inner part of the submarginal region were translucent. The submarginal region was about 75 to 150 μm wide and appeared denser than the surrounding upper layer. After 30 to 60 min of reincubation the wounds began to close by global movements of the upper layer (Fig. 4A).

Time-lapse videomicrography (n= 55)

We followed 55 wounded blastoderms with time-lapse videomicrography (Fig. 4B). During incubation the shape of the wound edge changed: from round to oval, sometimes even splitlike to round again. During most of the closure the edge was neither smooth nor perfectly round. The wounds closed by irregular movements of the submarginal region cells towards the wound like sheep in a flock: individual cells moved in different directions, globally the upper layer moved towards the wound. At the wound edge, individual cells and cell groups might herniate into the wound. The individual movements of cells and cell groups are not visible on still images.

Light microscopy (n= 203) and TEM (n= 54)

We studied 203 wounded blastoderms with light microscopy and 54 with TEM. The wounds were fixed after various reincubation times.

With light microscopy, the upper layer appears as a pseudostratified columnar epithelium in which most of the nuclei are situated halfway between the dorsal and ventral side. Unlike classical pseudostratified columnar epithelia (Krstic, 1984), cell divisions occur at the dorsal side, by a process called interkinetic migration, first described by Sauer (1936). We confirm the observations of Chen (1932) in the duck and Derrick (1937) in the chick,

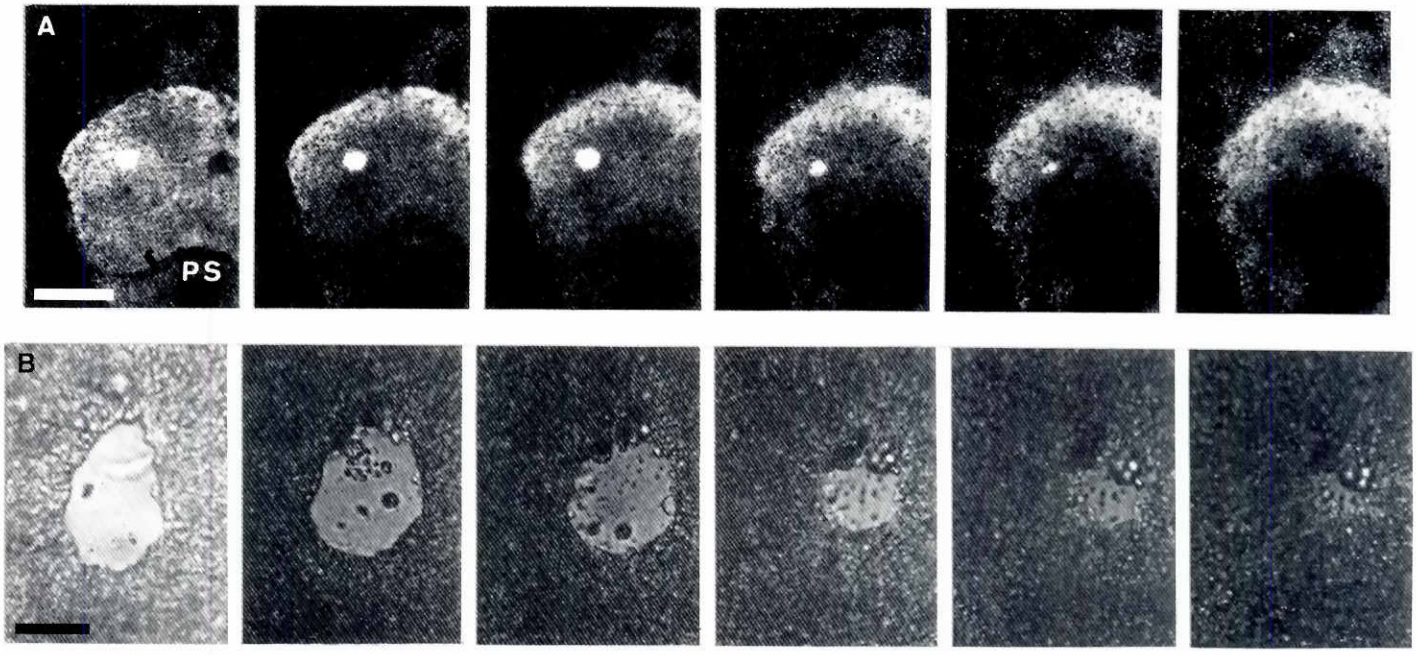

Fig. 4. Series of still images from time-lapse videography and videomicrography of closing wounds. *The ventral side of the upper layer faces the observer and anterior is up. The first image in each series is taken at reincubation. The succeeding time indications refer to the time after reincubation. The more translucent area in Fig. 4 A is the bare upper layer.* **(A)** *Videography: 0', 14', 44', 1 h 44', 2 h 14', 2 h 54'. PS: primitive streak. Bar, 0.5 mm.* **(B)** *Videomicrography: 0', 24', 1 h 04', 1 h 24', 1 h 35', 1 h 44'. Bar, 100 μm.*

TABLE 1

METAPHASE PLUS ANAPHASE COUNTS

Nr	h:min	Wound area M + A	Wound area n	Mirror image area M + A	Mirror image area n
1	4:00	8	234	6	197
2	5:30	3	95	7	87
3	5:00	3	110	3	76
4	5:15	24	518	15	363
5	7:15	7	173	8	108
6	7:00	7	133	3	86
7	7:30	19	354	14	257
8	6:00	10	194	10	174
9	3:00	9	168	8	128
10	3:00	2	40	4	48
11	2:15	5	114	1	77
12	2:00	3	100	10	68
13	2:00	4	77	5	84
14	2:15	3	69	4	73
15	2:15	8	278	7	215
16	2:15	4	152	3	113
17	2:00	0	40	0	42
18	2:45	8	221	17	190
19	2:45	4	29	2	22
20	2:45	3	48	1	50
21	2:15	11	371	18	347
22	2:15	5	187	13	213
Σ		150	3705	159	3018

Nr= experiment number; h:min= time between wounding and fixing; M= metaphase; A= anaphase; n= sum of all scored nuclei.

that most of the mitotic figures are oriented parallel to the upper layer.

There was no significant difference in the metaphase plus anaphase counts of the wound submarginal region and of the mirror image area, situated at the opposite side of the wound with respect to the primitive streak (Fig. 2) (Table 1) (paired t-test, $p<0.05$).

There was no significant difference either in the S-phase counts of the wound submarginal region and of the mirror image area (Table 2) (paired t-test, $p< 0.05$).

With TEM, cell debris and yolk particles were found inside the wounds. The epithelial structure of the wound submarginal region did not change throughout closure. The wound submarginal region was thicker than the upper layer around it. Fewer and smaller intercellular spaces were seen in the wound submarginal regions than in intact upper layer (Vanroelen and Vakaet, 1984). This explains why the number of nuclei in the wound submarginal regions was higher than in the mirror image area in Tables 1 and 2. The wound submarginal regions could be slightly curled ventrally. The basal side of the submarginal region cells showed numerous blebs throughout closure (Figs. 5 and 6A). Bundles of microfilaments were not only present at the apical side of the submarginal region cells (not shown), but also at the basal side, parallel to the cell membrane (Fig. 6B). We looked for a ring of microfilaments around wounds sectioned parallel to the wound edge. We found no indication of a continuous ring of intercellular microfilament bundles. The basal lamina at the edge of the wounds was absent throughout closure (Fig. 6C). Close to the wound edge the lamina densa was often wrinkled like a bunch of hairy cords, resembling the structures described by Leblond and Inoué (1989). At some places the lamina densa was loose from the basal side of the wound edge

TABLE 2

S-PHASE COUNTS

Nr	h:min	Wound area -	Wound area +	Wound area n	Mirror image area -	Mirror image area +	Mirror image area n
1	5:30	132	226	358	98	132	230
2	4:30	50	136	186	34	131	165
3	3:30	36	48	84	31	35	66
4	2:30	106	125	231	77	103	180
5	2:30	162	191	353	88	141	229
6	1:30	219	218	437	121	240	361
Σ		705	944	1649	449	782	1231

Nr= experiment number; h:min= time between wounding and fixing; += marked nuclei; -= non marked nuclei; n= sum of all scored nuclei.

cells without visible trauma to these cells. Closed wounds never left scars.

SEM (n= 38; 18 ventral aspect, 20 dorsal aspect)

With SEM 38 wounded blastoderms of various reincubation times were studied. The ventral aspect of the wounds was studied in 18 blastoderms. Inside the wounds we could find cell debris and yolk but never a basal lamina (Figs. 7 and 8). At the wound edge the basal lamina was absent and the basal side of individual cells could be seen. Below the wound submarginal region the basal lamina was visible, perforated by blebs at many points (Fig. 7). More blebs were seen at the wound submarginal regions than farther away from the wounds and at the ventral side of intact upper layer in similar areas. Fibrils were present in the pattern described by Wakely and England (1979), although interrupted at the wounds.

The dorsal aspect of the wounds was studied in 20 blastoderms. The wound submarginal region was clearly distinct, especially in young wounds (Fig. 8). Connecting cords were found, crossing at least one and up to four cells (Bellairs and Bancroft, 1975; Everaert *et al.*, 1988) (Fig. 8C).

Discussion

Contrary to the statement of Gallera and Castro-Correia (1960), experimental excision-wounds in the bare upper layer of the gastrulating chick blastoderm could close without having been recovered with deep layer.

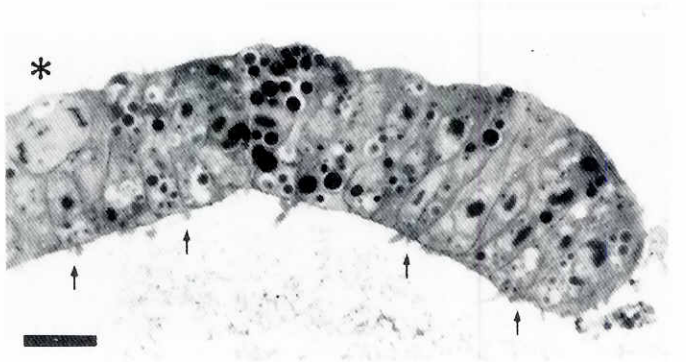

Fig. 5. Wound submarginal region; stage 4V; 15 minutes after reincubation; Kühn-staining. *The submarginal region is curled ventrally, intercellular spaces are rare, blebs (arrows), anaphase (asterisk). Bar, 10 μm.*

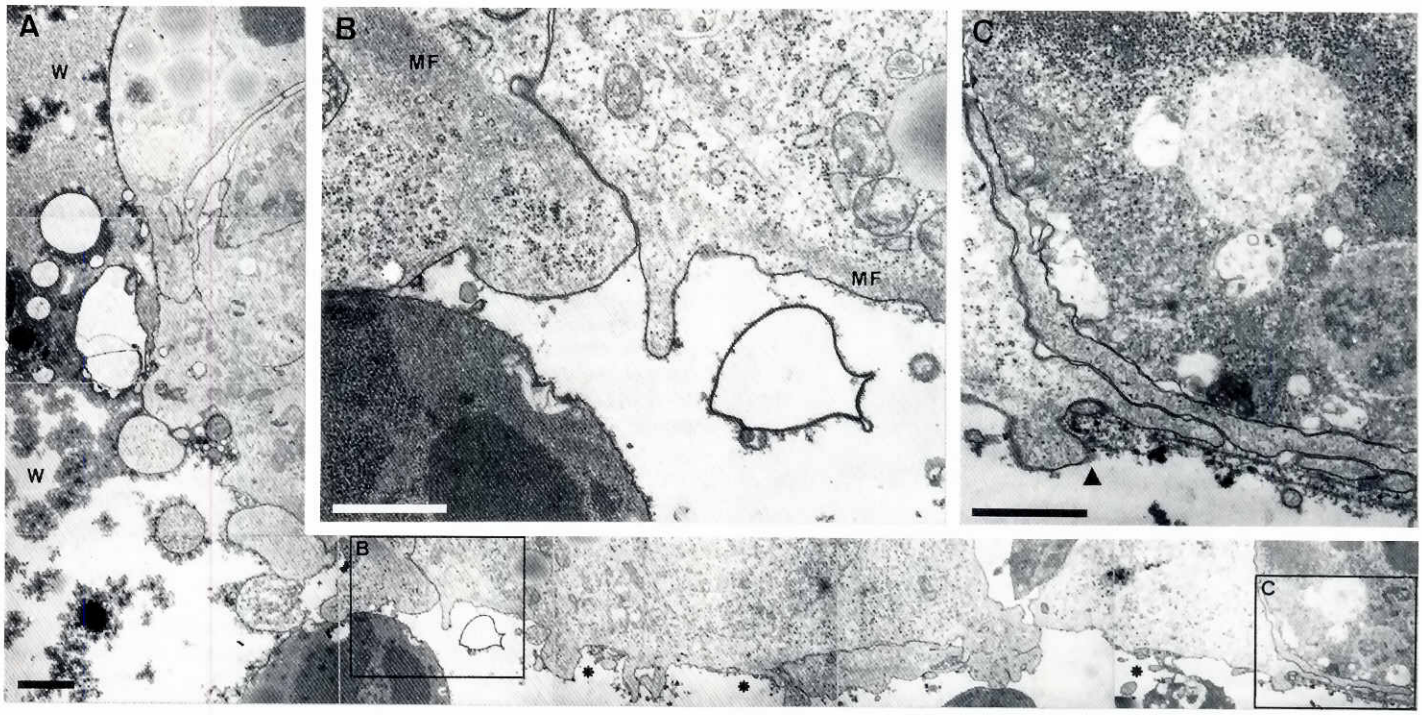

Fig. 6. TEM montage of a closing wound submarginal region; stage 6V; 2 hours after reincubation. (A) *Wound with wound fluid (W, W) at the left, fragments of lamina densa (asterisks). Bar, 3 μm.* **(B)** *(Boxed in A): microfilament bundles (MF) at the ventral side of the submarginal region cells. Bar, 5 μm.* **(C)** *(Boxed in A): edge of lamina densa (arrowhead). Bar, 5 μm.*

In wound closure there is no enhanced mitotic activity, as the metaphase plus anaphase index and the S-phase index in the wound submarginal region and the mirror image area do not differ significantly.

There is no migration on a substratum either, as there is no substratum inside the wounds. The wound edge and submarginal region are suspended in the fluid which leaks through the wound. Dorsally, the vitelline membrane that separates the blastoderm from the culture medium is not in contact with the wound edge and submarginal region (Fig. 3A). Ventrally, the deep layer is removed and no other cell layers are present (Figs. 2B and 3B).

Gabbiani *et al.* (1978) postulate that adult skin healing occurs by epidermal cell migration together with contraction of granulation fibroblasts in the underlying tissue. Martin and Lewis (1992) found an actin cable in the basal cells at the free edge of the wound in the chick wing bud epidermis. They proposed the contraction of an actin purse string as the healing mechanism. However, the actin cable they found was not continuous and moreover, the presence of it does not necessarily mean contraction. Indeed, Theriot and Mitchison (1991) have shown that actin polymerization may be propulsive. If in wound closure in the bare upper layer a purse string contraction were the main mechanism, one would expect the wound edge to present a steady circular aspect. With time-lapse videomicrography we saw that during closure the shape of the wound edge changed continuously and that cells and cell groups might herniate into the wound. Although we also observed microfilament bundles (Fig. 6B), we see their presence rather as a reaction against the surrounding fluid. Microfilaments might also play a role in the viscoelastic reaction at wounding (Sato *et al.*, 1987).

Time-lapse videography showed us that the wounds closed by global movements of the upper layer towards the wound. With videomicrography we saw that the cells of the wound submarginal region moved like sheep in a flock towards the wound. This agrees with the observations of Lash in Urodeles (1955). He deduced from displacements of marked cells that wounds close by the movement of the layer as a whole and by individual movements of cells to one another. Fristrom and Fristrom (1975) used the term cell rearrangement to describe cell shape changes observed with SEM during evagination of imaginal discs in *Drosophila melanogaster*. Fristrom (1976) defines rearrangement as small movements of many cells while close associations between neighboring cells are maintained. Keller (1978) observed cell rearrangement with time-lapse cinemicrography in the superficial layer of *Xenopus laevis*. Fristrom (1988) concludes in a review on the cellular basis of epithelial morphogenesis «that we do not yet know what cells do in order to rearrange».

We propose a novel mechanism for the global movement of the upper layer towards the wound and for the movements of individual wound submarginal region cells. With videomicrography we saw that the upper layer cells move in respect to one another. We think that, at their basal side, they are not confined to a specific part of the basal lamina. We propose that during wound closure the movements of the cells are driven by mitotic pressure, by which we mean the horizontal pressure exerted by the addition of daughter cells and by the parting of the anaphase nuclei and telophase cells. This moving apart brings about changes in the relative position of neighbor cells. Connecting cords bridging one or more apical cell surfaces are witnesses of these movements, as in chick blastoderms most of the mitotic figures are lying at the dorsal side of the upper layer and parallel to it (Fig. 6). The individual movements of cells result in a global movement of the upper layer towards the wound, where there is no mitotic pressure, as there are no cells within the wound.

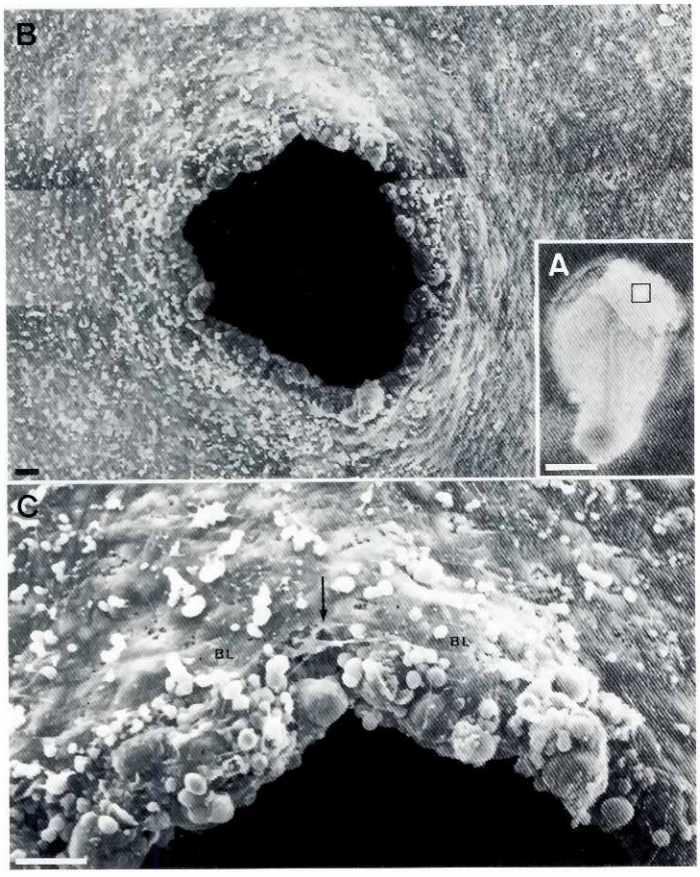

Fig. 7. Ventral side of a wound; stage 5V; no reincubation. (A) *Polaroid photograph before fixation, bar: 0.5 mm.* **(B)** *(Boxed in A): SEM montage, blebs perforating the basal lamina especially close to the wound. Bar, 10 μm.* **(C)** *Wound submarginal region and edge: basal lamina (BL) ends below the submarginal region cells (arrow). Bar, 10 μm.*

We extend the proposed mechanism to the normal movements of Anlage Fields during gastrulation. A wound and a primitive streak resemble each other histologically. The submarginal regions of closing wounds and the lips of the primitive streak are thicker than the upper layer around them. Both the wound submarginal region and the groove of the primitive streak curl ventrally and are thinner at their extremities. The basal lamina is interrupted at the primitive groove (Bortier *et al.*, 1989) and at the wound edge. The movements in the upper layer towards a wound and towards the primitive streak are similar when observed with time-lapse videomicrography: global movements of the upper layer in which individual cells move like sheep in a flock. The movements in the upper layer to close a wound and the movements in the Anlage Fields during gastrulation might be driven by the same mechanism: mitotic pressure. These movements might be orientated towards a sink in mitotic pressure due to a wound or to ingression at the primitive streak.

Materials and Methods

Materials

Chick eggs (White Rock, from the Rijksstation voor Pluimveeteelt, Merelbeke, Belgium) and quail eggs (*Coturnix coturnix* japonica, from laboratory stock) were incubated at 38°±0.5°C for 15 hours to obtain stage 4V to 6V blastoderms (Vakaet, 1970). They were cultured by New's (1955) technique, except that the culture medium used was a mixture of 25 ml thin egg white and a gel made of 150 mg Bacto-agar Difco (Detroit, Michigan, USA) in 25 ml Ringer's solution. This semisolid medium allowed microsurgery as well as further culturing.

Experimental excision-wounding

Tungsten needles were used to detach the marginal hypoblast from the yolk endoderm at the periphery of the proamnion (endophyll wall) without touching the upper layer (Fig. 9). The anterior part of the deep layer, the endophyll wall and the hypoblast, were reclined and discarded. In a first series of experiments a fragment of the upper layer (approximately 0.25x0.25 mm) was removed using tungsten needles. It was impossible to cut these wounds without making lacerations at the angles, involving sometimes abortion of the blastoderm. Therefore the wounding procedure was modified (Bortier, 1987; Bortier and Vakaet, 1987). We used a Ringer-filled Pasteur pipette with a tip diameter of 0.20 mm to 0.25 mm. From it, a drop was pushed out just before touching the upper layer. A more or less circular fragment (Figs. 7 and 8) of the upper layer was aspiration-punched and discarded. During this procedure the vitelline membrane was left intact. One wound was made per blastoderm. The original diameter of the wound could vary even when the same pipette was used. After the intervention a Polaroid photograph was taken using an M8 zoom stereomicroscope (Wild, Heerbrugg, CH-9435) at magnification x25. The culture vessels were covered with a glass lid, sealed with melted paraffin and further incubated at 38°±0.5°C. The blastoderms were observed regularly in an incubator adapted to follow several culture vessels without temperature loss. Observation of the wounds was documented regularly with Polaroid photography.

Time-lapse videography

For time-lapse videography (Bortier and Vakaet, 1992b) we used an M8 stereomicroscope (Wild, Heerbrugg, CH-9435), on top of which a WV-1850 camera (Panasonic, Osaka, Japan) was mounted in a plexiglass incubator at 38±1°C. The camera was linked to a U-matic video recorder VO-5850P

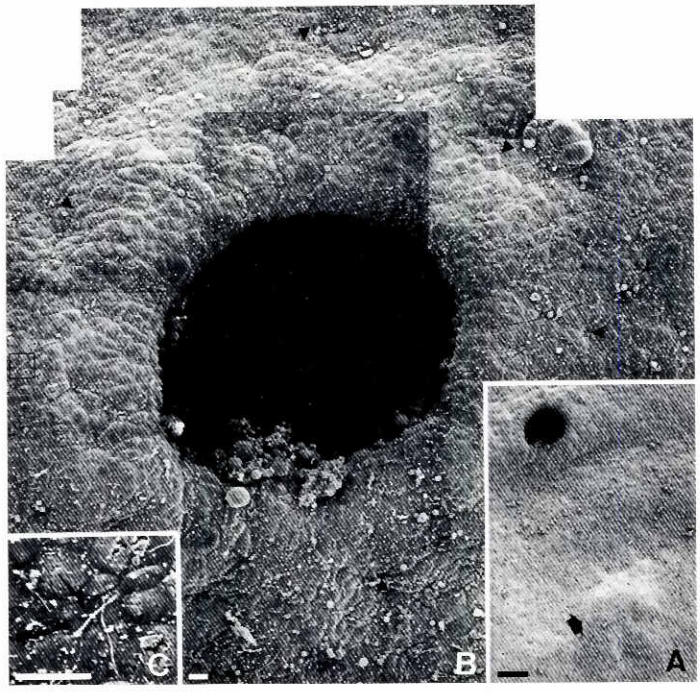

Fig. 8. SEM of the dorsal aspect of a wound; stage 4V; 1 hour after reincubation. (A) *Overview: Hensen's node (broad arrow). Bar, 100 μm.* **(B)** *Montage, wound submarginal region periphery (arrowheads). Bar, 10 μm.* **(C)** *(Boxed in B): connecting cords (arrow pointing to one midbody). Bar, 10 μm.*

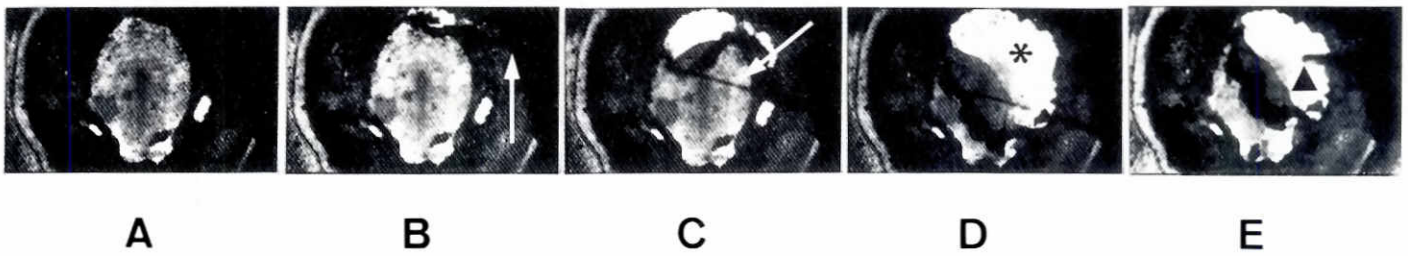

Fig. 9. Still images of experimental excision-wounding. *The ventral side of the blastoderm faces the observer; anterior is up (see Fig. 1 for details).* **(A)** *Blastoderm on the vitelline membrane.* **(B)** *Loosening of the deep layer at the endophyll wall with a tungsten needle (arrow).* **(C)** *Reclining the endophyll wall and the hypoblast (arrow).* **(D)** *Bare upper layer in the proamnion (asterisk).* **(E)** *Aspiration-punching of a wound in the bare upper layer with a Pasteur pipette (arrowhead).*

(Sony, Tokyo, Japan) through an animation control unit EOS AC580 (EOS Electronics A.V., Barry, South Glamorgan). A time date generator WJ-810 (Panasonic, Osaka, Japan) displayed the chronological information on a WV-5340 monitor (Panasonic, Osaka, Japan). One video-image was recorded every 30 seconds, yielding an acceleration of x750 at normal projection speed (25 images/second).

Time-lapse videomicrography

For time-lapse videomicrography (Bortier and Vakaet, 1989) (Fig. 10) we used an invert microscope (Fluovert, Leitz, Wetzlar, Germany) with Nomarski optics (obj. NPL Fluotar L 40/0.60). An AVEC (Allen's Video Enhanced Contrast)/VIM (Video Intensified Microscope) system C-1966-01 (Hamamatsu Phototonics, Hamamatsu-City, Japan) was linked to a U-matic video recorder VO-5850P (Sony, Tokyo, Japan) in an incubator at 38°±1°C. One video-image was recorded every 18 seconds, yielding an acceleration of x325 in projection. Experiment data were recorded on videocassette using a videotypewriter VTW-210 (FOR.A, Japan). Still images were photographed from the video screen with a Leicaflex SL (Leitz, Wetzlar, Germany) (obj. Makro-Elmarit 1:2.8/50) on Technical Pan film 15 din (Kodak). Exposure was at least 1 second.

Light microscopy, mitotic activity and TEM

For light microscopy the reincubation of wounded blastoderms was interrupted by fixing them for two hours in a mixture of absolute ethanol, formaldehyde 4%, acetic acid (75:20:5, v:v:v). After paraffin embedding and sectioning they were stained after Feulgen and Rossenbeck (1924).

The metaphase plus anaphase figures were scored on every third serial section to avoid counting the same cells twice. At the wound submarginal region a strip of 65 µm (measured with a calibrated eyepiece) on the medial and lateral side of the wound was determined. A control area of 2x65 µm was scored in the mirror image area. The counts were made in duplo by two independent observers.

The S-phase counts were scored in the same way as the metaphase and anaphase figures, but after autoradiography. To that end wounded blastoderms were transferred to a culture medium to which 10 µC/ml ^{3}H-thymidine (specific radioactivity: 5 C/mmol, Amersham, Buckinghamshire, UK) was added. After 30 minutes of incorporation at 38°C the blastoderms were fixed and sectioned as described. The Feulgen-stained sections were dipped in Ilford L4 emulsion (Ilford Nuclear Research Emulsion, Ilford Limited, Essex, UK). The optimal exposition time was determined by trial developments after 3 to 6 weeks. Finally the sections were developed in Ilford L4 developer and fixed in sodium thiosulfate 3% in water (w/v).

For TEM the wounded blastoderms were fixed for 2 hours in the mixture described by Vanroelen and Vakaet (1981), consisting of a solution of 1% glutaraldehyde (v:v) and 1% tannin (w:v) in sodium cacodylate buffer 0.1 M at pH 7.4. The blastoderms were postfixed for 30 minutes in 1% OsO_4 in the same buffer (w:v), dehydrated in an ethanol gradient and embedded in LX-112 epoxy resin (Ladd Research Industries, Inc., Burlington, VT, USA). Three series of consecutive 2 µm sections of the wound submarginal region were disposed alternately on three object glasses. One series was stained with 1% toluidine blue in distilled water (w:v) for 50 seconds at 70°C. Another series was stained according to Kühn (1970), a combination of periodic acid Schiff-Hotchkiss and toluidine blue. At the level of the wounds, series of 2 µm sections were alternated with series of 50-60 nm sections, made with an Ultratome Nova (Reichert-Jung, Vienna, Austria). They were stained during 30 minutes at 40°C with uranylacetate and during 15 minutes at 20°C with lead citrate in an Ultrostainer 2168 (concentrations not communicated) (LKB, Bromma, Sweden) and examined with a Jeol-100B TEM microscope operated at 80 kV. Photographs were made on Electron Microscopy film 4489 Estar Thick Base (Kodak). Montages were made of selected areas.

SEM

For SEM the blastoderms were fixed for 2 hours in 1% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4) (v/v). They were postfixed for 30 minutes in 1% OsO_4 in sodium cacodylate buffer (0.1 M, pH 7.4) (w/v) and dehydrated in an ethanol gradient up to ethanol 70°. They were further dehydrated in acetone and dried in a critical point dryer (Balzers CPD 030, FL, USA) with CO_2 as transition fluid. The specimens were mounted on an aluminium stub with Silver Print (GC Electronics, Rockford Ill) and coated with a 20 nm gold layer in an E5000 sputter coater (Polaron, Biorad House, UK). Montages of the wounds photographed in a MSI SR-50 scanning electron microscope at 10 kV on 5-TMX 120 film (Kodak) were made of every blastoderm studied.

Acknowledgments

This work was supported by a grant of the National Fund for Scientific Research (N.F.S.R.) (3.9001.87), Brussels, Belgium. H. Bortier is a Senior Research Assistant to the N.F.S.R., Brussels, Belgium. The authors thank R. De Smedt, M. De Vrieze, P. Martens, R. Mortier, E. Roosen, G. Van Limbergen, E. Van Meirhaeghe, L. Van Trappen and N. Verweire for excellent assistance.

References

BELLAIRS, R. and BANCROFT, M. (1975). Midbodies and beaded threads. *Am. J. Anat. 143:* 393-398.

BORTIER, H. (1987). Wound healing in the upper layer of the chicken blastoderm. *Eur. J. Cell Biol. 44 (Suppl.):* 34 (Abstr.).

BORTIER, H. and VAKAET, L. (1987). Wound healing in the upper layer of the chicken blastoderm. *Cell Differ. 20 (Suppl.):* 114 (Abstr.).

BORTIER, H. and VAKAET, L. (1989). Videomicrography with Nomarski optics and AVEC-system of wound closure in the upper layer of the chicken blastoderm. *Cell Differ. Dev. 27 (Suppl.):* 101 (Abstr.).

BORTIER, H. and VAKAET, L. (1992a). Mesoblast anlage fields in the upper layer of the chicken blastoderm at stage 5V. In *Formation and Differentiation of Early Embryonic Mesoderm* (Eds. R. Bellairs *et al.*). NATO ASI Series A: Life Sciences, Vol. 231. Plenum Press, New York, pp. 1-7.

BORTIER, H. and VAKAET, L.C.A. (1992b). Fate mapping the neural plate and the intraembryonic mesoblast in the upper layer of the chicken blastoderm with xenografting and time-lapse videography. *Development 1992 (Suppl.).* The Company of Biologists Limited, Cambridge, pp. 93-97.

BORTIER, H., DE BRUYNE, G., ESPEEL, M. and VAKAET, L. (1989). Immunohistochemistry of laminin in early chicken and quail blastoderms. *Anat. Embryol. 180:* 65-69.

CALLEBAUT, M. (1982). Labelling of the epigerminal space on quail blastoderms after injection of 3H-leucine to the mother. *IRCS Med. Sci. 10:* 248.

CALLEBAUT, M. (1983). Autoradiographic demonstration of the penetration of albumen-derived material through the vitelline membrane into the egg yolk, exterior to the avian blastoderm. *Poultry Sci. 62:* 1657-1659.

CHEN, B.K. (1932). The early development of the duck's egg, with special reference to the origin of the primitive streak. *J. Morphol. 53(1):* 133-179.

DERRICK, G.E. (1937). An analysis of the early development of the chick by means of the mitotic index. *J. Morphol. 61:* 257-284.

EVERAERT, S., ESPEEL, M., BORTIER, H. and VAKAET, L. (1988). Connecting cords and morphogenetic movements in the quail blastoderm. *Anat. Embryol. 177:* 311-316.

FEULGEN, R. und ROSSENBECK, H. (1924). Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. *Hoppe Seylers Z. Physiol. Chem. 135:* 203-252.

FRISTROM, D. (1976). The mechanism of evagination of imaginal discs of *Drosophila melanogaster.* III. Evidence for cell rearrangement. *Dev. Biol. 54:* 163-171.

FRISTROM, D. (1988). The cellular basis of epithelial morphogenesis. A review. *Tissue Cell 20 (5):* 645-690.

FRISTROM, D. and FRISTROM, J.W. (1975). The mechanism of evagination of imaginal discs of *Drosophila melanogaster.* I. General considerations. *Dev. Biol. 43:* 1-23.

GABBIANI, G., CHAPONNIER, C. and HUTTNER, I. (1978). Cytoplasmic filaments and gap junctions in epithelial cells and myofibroblasts during wound healing. *J. Cell Biol. 76:* 561-568.

GALLERA, J. and CASTRO-CORREIA, J. (1960). Technique assurant la cicatrisation des excisions pratiquées dans l'aire pellucide de jeunes blastodermes de poulet et la possibilité d'obtenir des inductions neurales dans l'ectophylle de l'aire vasculaire. *C.R. Séa. Soc. Biol. CLIV (10):* 1728-1732.

IHARA, S., MOTOBAYASHI, Y., NAGAO, E. and KISTLER, A. (1990). Ontogenetic transition of wound healing pattern in rat skin occurring at the fetal stage. *Development 110:* 671-680.

JAFFE, L.F. and STERN, C.D. (1979). Strong electrical currents leave the primitive streak of chick embryos. *Science 206:* 569-571.

KELLER, R.E. (1978). Time-lapse cinemicrographic analysis of superficial cell behavior during and prior to gastrulation in *Xenopus laevis. J. Morphol. 157:* 223-248.

KRSTIC, R.V. (Ed.) (1984). *Illustrated Encyclopedia of Human Histology.* Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.

KÜHN, C. (1970). Application de techniques de colorations utilisées en microscopie optique, aux coupes semi-fines de microscopie électronique. *Med. Lab. 23:* 204-216.

LASH, J.W. (1955). Studies on wound closure in Urodeles. *J. Exp. Zool. 128:* 13-27.

LEBLOND, C.P. and INOUE, S. (1989). Structure, composition, and assembly of basement membrane. *Am. J. Anat. 185:* 367-390.

MAREEL, M.M. and VAKAET, L.C. (1977). Wound Healing in the primitive deep layer of the young chick blastoderm. *Virchows Arch. B 26:* 147-157.

MARTIN, P. and LEWIS, J. (1992). Actin cables and epidermal movement in embryonic wound healing. *Nature 360:* 179-183.

NEW, D.A.T. (1955). A new technique for the cultivation of the chick embryo *in vitro. J. Embryol. Exp. Morphol. 3(4):* 326-331.

PEACOCK, E.E. (Ed.) (1985). *Wound Repair.* W.B. Saunders Inc., Philadelphia.

SATO, M., SCHWARZ, W.H. and POLLARD, T.D. (1987). Dependence of the mechanical properties of actin/α-actinin gels on deformation rate. *Nature 325:* 828-830.

SAUER, F.C. (1936). The interkinetic migration of embryonic epithelial nuclei. *J. Morphol. 60(1):* 1-11.

STANISSTREET, M., WAKELY, J. and ENGLAND, M.A. (1980). Scanning electron microscopy of wound healing in *Xenopus* and chicken embryos. *J. Embryol. Exp. Morphol. 59:* 341-353.

STERN, C.D., MANNING, S. and GILLESPIE, J.I. (1985). Fluid transport across the epiblast of the early chick embryo. *J. Embryol. Exp. Morphol. 88:* 365-384.

THERIOT, J.A. and MITCHISON, T.J. (1991). Actin microfilament dynamics in locomoting cells. *Nature 352:* 126-131.

THEVENET, A. (1984). Proliferation cellulaire au cours de la cicatrisation du tegument d'embryon de poulet de 5 jours. *Arch. Anat. Microsc. 73(2):* 121-132.

VAKAET, L. (1970). Cinephotomicrographic investigations of gastrulation in the chick blastoderm. *Arch. Biol. (Liège) 81(3):* 387-426.

VANROELEN, Ch. and VAKAET, L. (1981). The effect of the osmolality of the buffer solution of the fixative on the visuatlization of microfilament bundles in the chick blastoderm. *J. Submicrosc. Cytol. 13:* 89-93.

VANROELEN, Ch. and VAKAET, L. (1984). The influence of tissue handling before fixation on the morphology of the chick blastoderm. *J. Microsc. 134 (2):* 173-176.

WAKELY, J. and ENGLAND, M.A. (1979). Scanning electron microscopical and histochemical study of the structure and function of basement membranes in the early chick embryo. *Proc. R. Soc. Lond. [Biol.] 206:* 329-352.

WEISS, P. and MATOLTSY, A.G. (1959). Wound healing in chick embryos *in vivo* and *in vitro. Dev. Biol. 1:* 302-326.

Accepted for publication: June 1993