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Differentiation

Induction of keratinocyte type-I transglutaminase in epithelial cells of the rat

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Abstract. Using immunogold-silver techniques, we have demonstrated that, in rats, type-I (keratinocyte) transglutaminase is expressed primarily in stratified squamous epithelia of the integument, the upper digestive tract, and the lower female genital tract. In these epithelia, the enzyme was found to be present predominantly in the granular layer, but was evident at low levels even in the basal layer, especially in the genital tract. No immunoreactivity was detected in glandular, columnar, or transitional epithelia or in soft tissues. However, considerable enzyme antigenicity was observed in the endometrium and in major ducts of the pancreas and mammary glands of near-term pregnant and early postpartum females. In cultures, substantial immunoreactivity was readily identifiable not only in epidermal, vaginal, and esophageal epithelial cells (immunopositive in vivo), but also in urinary bladder, seminal vesicle, and tracheal epithelial cells (immunonegative in vivo). Primary epithelial outgrowths from bladder and seminal vesicle tissue explants were immunopositive, demonstrating rapid adaptation to the culture environment. These results reveal three distinct levels of regulation of transglutaminase expression in various cell types: (1) during the differentiation of keratinocytes, (2) during pregnancy, being evident principally in the endometrium but detectable elsewhere as well, and (3) during the cultivation of certain epithelia which do not normally express the enzyme in vivo. We conclude that type-I transglutaminase may be a valuable marker for elucidating the regulation of normal epithelial differentiation and squamous metaplasia.

Introduction

To understand the regulation of complex differentiation processes, the identification of marker enzymes and structural proteins whose expression can be examined in detail is critical. Analyzing determinants of their expression in a spectrum of tissues may then permit elucidation of the complicated overlapping regulation responsible for maintaining differentiated function. Maturing keratinocytes of stratified squamous epithelia undergo striking morphological and biochemical changes, including the accumulation and altered expression of keratin tonofilaments, digestion of cellular organelles, and formation of rigid squames ensheathed by cross-linked protein envelopes [18]. This last feature of maturation, which is seen both in vivo and in culture, is mediated by the enzyme, transglutaminase [39], which catalyzes the formation of ε-(γ-glutamyl)-lysine cross-links between proteins. Recent characterization of the enzyme, which is largely membrane-bound in cultured keratinocytes [24, 45, 48], has suggested that this localization is important for the formation of envelopes at the cell periphery. Monoclonal antibodies raised against the particulate enzyme of cultured human keratinocytes permit its immunoprecipitation as a 92-kilodalton (kd) protein [48]. Being expressed in human epidermis but not dermis, immunochemically detectable transglutaminase reaches its maximum level in the upper spinous and granular cells, befitting its role in cross-linking of the envelope constituent, involucrin, which is expressed at a similar stage of epidermal maturation [48].

Many types of transglutaminase have been reported. They are secreted in plasma [4, 25, 30] and seminal fluid [51], expressed as soluble intracellular enzymes in peripheralblood cells [13, 36], lens [26], epidermis [5, 32], hair follicles [9, 20], and numerous other tissues [8], and expressed as particulate enzymes in rat lung [10, 19], lens [2], and liver [7, 46], as well as in cell lines from various sources [3]. These types constitute a family of distinct enzymes, as judged by their biochemical properties [16] and the regulation of their synthesis by physiological agents such as vitamin A, cyclic AMP, hydrocortisone, and calcium [13, 24, 42, 49]. Before their interrelationships and functions can be clarified, much remains to be learned concerning the tissue specificity, regulation, and biochemistry of transglutaminases, especially the particulate forms.

In addition to the particulate enzyme, cultured keratinocytes express an antigenically distinct transglutaminase that corresponds to the soluble 'tissue' form studied by Chung [8] and other investigators. In our initial work, we designated these as types I and II, respectively, i.e., in the order of their elution from DEAE-cellulose columns [48]. Earlier work did not resolve whether the expression of the type-I enzyme is limited to maturing stratified squamous epithelial cells or whether it is also responsible for the activities reported in certain other tissues. We chose laboratory rats for the present work due to the ready availability of tissues from which considerable biochemical information about transglutaminases is accumulating, and because a monoclonal antibody (B.C1) that we have raised to the particulate transglutaminase in human keratinocytes immunoprecipitates the corresponding rat enzyme [48].

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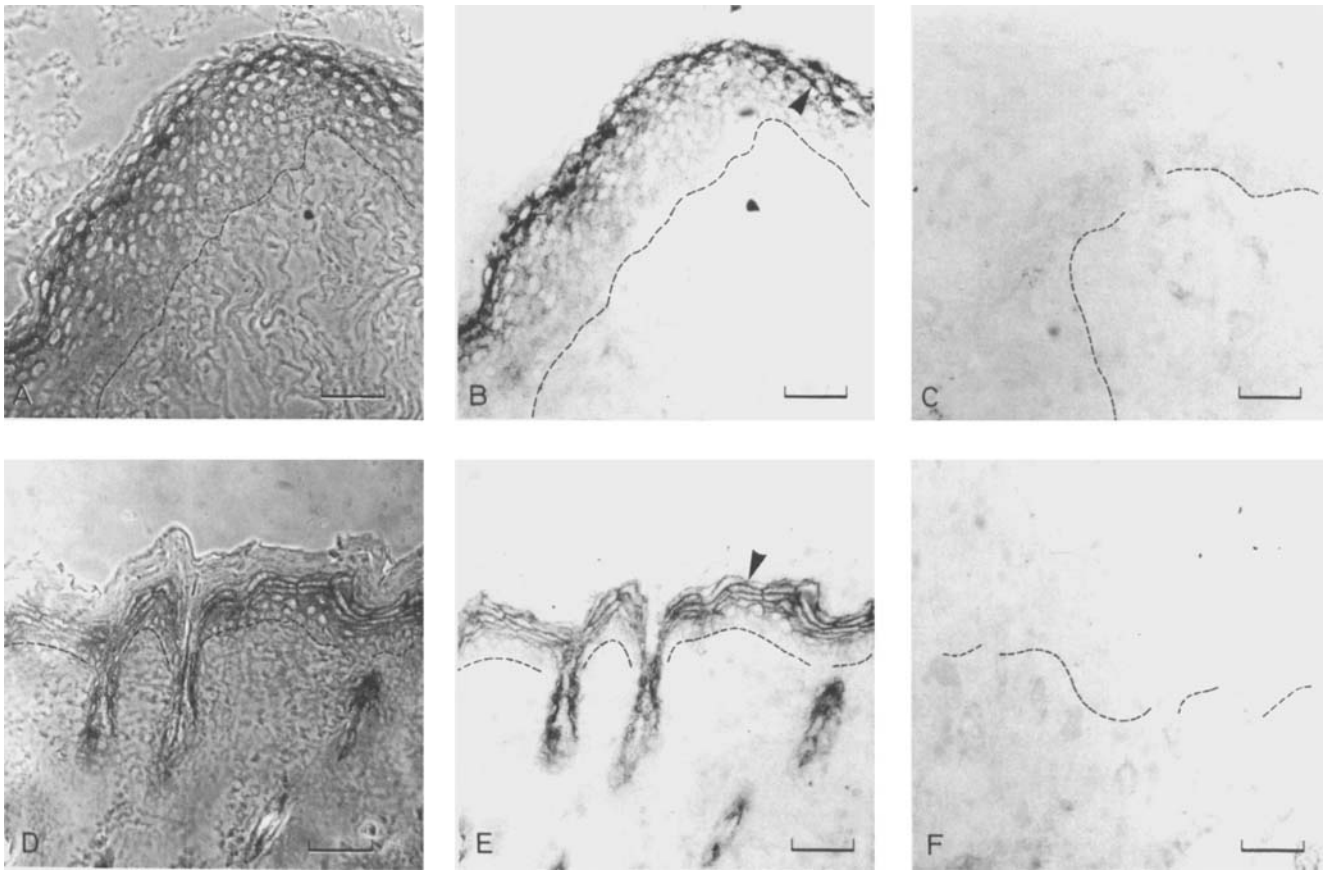

Fig. 1 A–F. Distribution of immunoreactivity in human and rat epidermis: human foreskin (**A–C**) and newborn rat skin (**D–F**). **A, D** Phase-contrast microscopy. **B** Same section as in **A** stained with three monoclonal antibodies to type-I transglutaminase. (The two additional human-specific monoclonal antibodies were used to enhance visualization of the enzyme in the more basal layers.) **E** Same section as in **D** stained with B.C1 monoclonal antibody to type-I transglutaminase. **C, F** Sections of human and rat skin, respectively, stained with control ascites fluid. Note that, in both the human and rat epidermis, the granular layer contains abundant transglutaminase that is primarily concentrated toward the cell periphery (*arrowheads* in **B** and **E**). In deeper cell layers, the transglutaminase distribution is more diffuse and less abundant, although no cell layer appears to be truly devoid of the enzyme. *Dotted lines* indicate the position of the basement membrane. *Bars*, 50 μm

Cells from the epithelia of numerous rat tissues can be readily grown in culture as continuous lines [21] using the 3T3 feeder layer technique [38]. Even cells from glandular and other nonkeratinized epithelia, however, show a remarkable convergence in properties toward a keratinocyte phenotype in culture [35]. In addition to a high keratin content and, commonly (but not in all cases), stratification with obvious squame formation, such cells display the ability to form cross-linked envelopes. In the present study, we report that this change of phenotype – resembling squamous metaplasia in vivo – includes the expression of the particulate keratinocyte transglutaminase, thus providing a potentially useful marker for elucidating this poorly understood process [27]. For such a purpose, involucrin also appears to be an excellent marker in humans [41, 50], but an antigenically or biochemically related protein has not been found in species lower than primates [40].

Methods

Immunogold-silver labeling. This was based on recent applications [12, 22]. Monoclonal antibodies BC.1, BD.4, and IB.3 [48] were purified from mouse ascites using a protein-A Sepharose (Pharmacia, Piscataway, NJ) column and were stored individually at concentrations of 0.5–3 mg/ml in Dulbecco's phosphate-buffered saline ($-Ca^{++}$, Mg^{++}) containing 0.2% NaN_3 and 5 mg/ml bovine serum albumin (BSA). Excised rat tissues (Sprague Dawley; Charles River, Wilmington, Mass) were embedded in Tissue-Tek OCT compound (Miles Laboratories, Elkhart, Ind), frozen in Eppendorf tubes in liquid N_2, and stored at −70° C until use. Cryostat sections (thickness, 2–6 μm) were air dried on glass slides previously coated with 0.5% chrome alum gelatin. The sections were incubated for 1 h with 100 μl purified antibody diluted 1:500 (B.D4, I.B3) or 1:1,000 (B.C1) in buffer consisting of 0.02 *M* Tris-HCl (pH 8.2), 0.9% NaCl, and 0.1% BSA containing 1% nonimmune goat serum, and then for 20 min in this buffer containing 5% goat serum. Slides containing sections of pancreas were first incubated for 1 min in phenylmethylsulfonyl fluoride (1 mg/ml in buffer). After treatment with the primary antibody, the sections were washed three times (these and subsequent washes were for 5 min) in buffer and incubated for 1 h with Auroprobe goat antimouse IgG coupled to colloidal gold (Janssen Life Sciences, Piscataway, NJ) diluted 1:80. Sections were given three washes in buffer and two washes in Dulbecco's phosphate-buffered saline ($-Ca^{++}$,

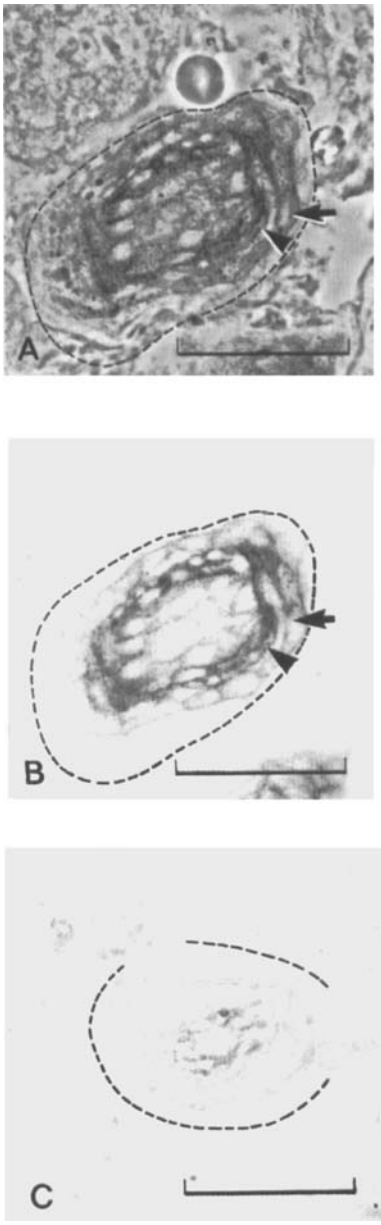

Fig. 2A–C. Distribution of immunoreactivity in rat hair follicles. **A** Phase-contrast microscopy. **B** Same section stained with monoclonal antibody B.C1 to type-1 transglutaminase (bright field). **C** Same specimen stained with control ascites fluid (bright field). Immunoreactivity is visible in both the outer root sheath (*arrows*) and inner root sheath (*arrowheads*) of rat hair follicles. *Dotted lines* indicate the periphery of the dermal sheath. *Bars*, 50 μm

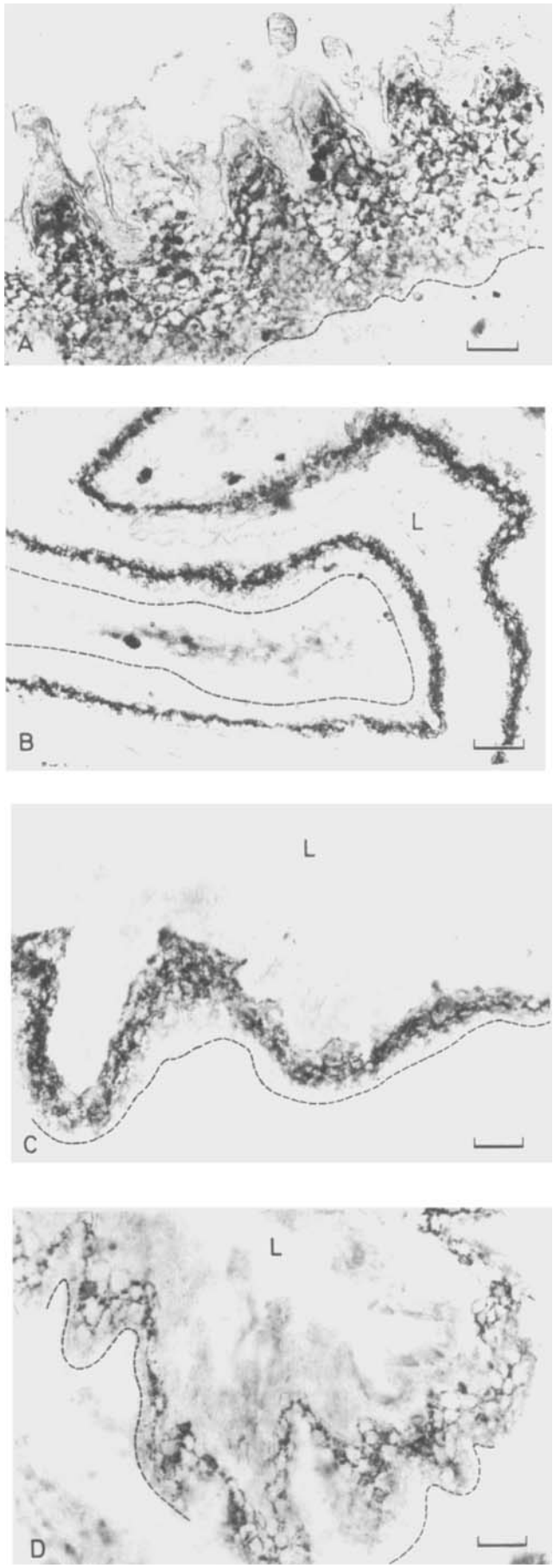

Fig. 3A–D. Distribution of immunoreactivity in stratified squamous epithelia of the rat digestive tract; immunogold-silver labeling with monoclonal antibody B.C1 against type-1 transglutaminase (bright-field microscopy). **A** Dorsal surface of the tongue; **B** esophagus; **C** forestomach; **D** stomach near the esophageal junction. *Dotted lines* indicate the position of the basement membrane; *L*, lumen. Note the punctate nature of the labeling in more superficial cell layers. In the tongue (**A**), immunoreactivity is clearly detected throughout the epithelium. It is not, however, detectable in the deeper layers of the esophageal epithelium (**B**), even though the cells immediately below the cornified layer exhibit rich staining. *Bars*, 50 μm

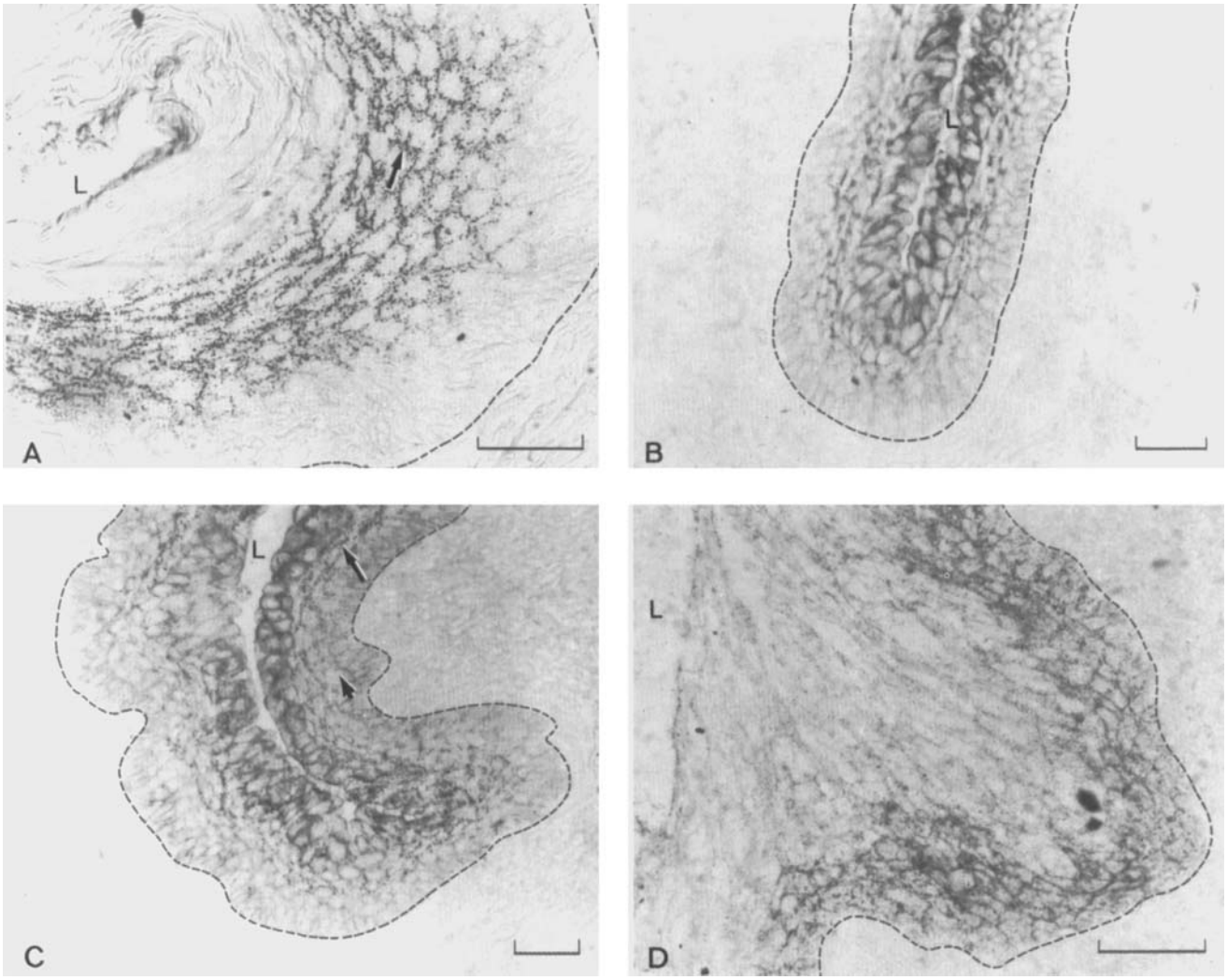

Fig. 4 A–D. Distribution of immunoreactivity in the vagina and cervix of rats; immunogold-silver labeling with B.C1 monoclonal antibody against type-I transglutaminase (bright-field microscopy). **A** Cervix during estrus; **B** cervix during early pregnancy; **C** vagina during early pregnancy; **D** vagina during late pregnancy. *Dotted lines* indicate the position of the basement membrane; *L*, lumen. Note the exclusively peripheral, punctate localization of the enzyme in the estrous cervix (*arrow* in **A**) and the absence of detectable enzyme in more basal epithelial layers. This is in contrast to the cervix and vagina during early pregnancy (**B, C**), where, although a small amount of punctate staining is visible (*arrow* in **C**), diffuse labeling is evident throughout the epithelium (*arrowhead*). *Bars*, 50 μm

Mg^{++}), and were fixed for 15 min in 2% glutaraldehyde (EM grade) in saline. The slides were then washed twice in saline, three times in distilled water (3 min each), and once (for 2 min) in 0.2 *M* citrate buffer, pH 3.85. For silver enhancement of the bound colloidal gold, the slides were incubated for 4–7 min in this citrate buffer containing 77 m*M* hydroquinone and 5.5 m*M* silver lactate, during which they were protected from light. In later experiments, silver enhancement was performed using the light-insensitive IntenSE method of Janssen. Following enhancement, the slides were treated with Janssen fixing solution for 2 min and washed in distilled water. Negative controls, which exhibited no detectable nonspecific binding, were performed by replacing the primary antibody with either partially purified NS-1 ascites fluid (Cappel/Worthington, Malvern, Pa) or with buffer alone. Negative controls were examined in parallel for each tissue, and positive controls were included on each slide. In lieu of sectioning, certain cultures were stained in dishes following fixation for 1 min with 0.1% formalin in saline, three washes in saline, and a 5-min treatment with 0.1% saponin in saline. The cultures were exposed to monoclonal antibody for 1 h prior to the above-mentioned immunogold and silver treatment.

Cell culture. Keratinocytes were cultivated with 3T3 feeder layer support according to standard procedures [1, 37] in Dulbecco-Vogt Eagle's and Ham's F-12 media (a 3:1 mixture) supplemented with 5% fetal bovine serum, 0.4 μg/ml hydrocortisone, 10 ng/ml epidermal growth factor, 9 ng/ml cholera toxin, 0.18 m*M* adenine [33], 20 p*M* triiodothyronine, 5 μg/ml insulin, 5 μg/ml transferrin, 0.1 mg/ml penicillin, and 0.1 mg/ml streptomycin. Rat epithelial cell cultures were initiated as explants with feeder layer support. Cells from the epithelial outgrowths were disaggregated by trypsinization and then serially passaged, with periodic removal of fibroblasts as necessary by vigorous rinsing with 0.5 m*M* ethylenediaminetetraacetate (EDTA) in isotonic saline [47].

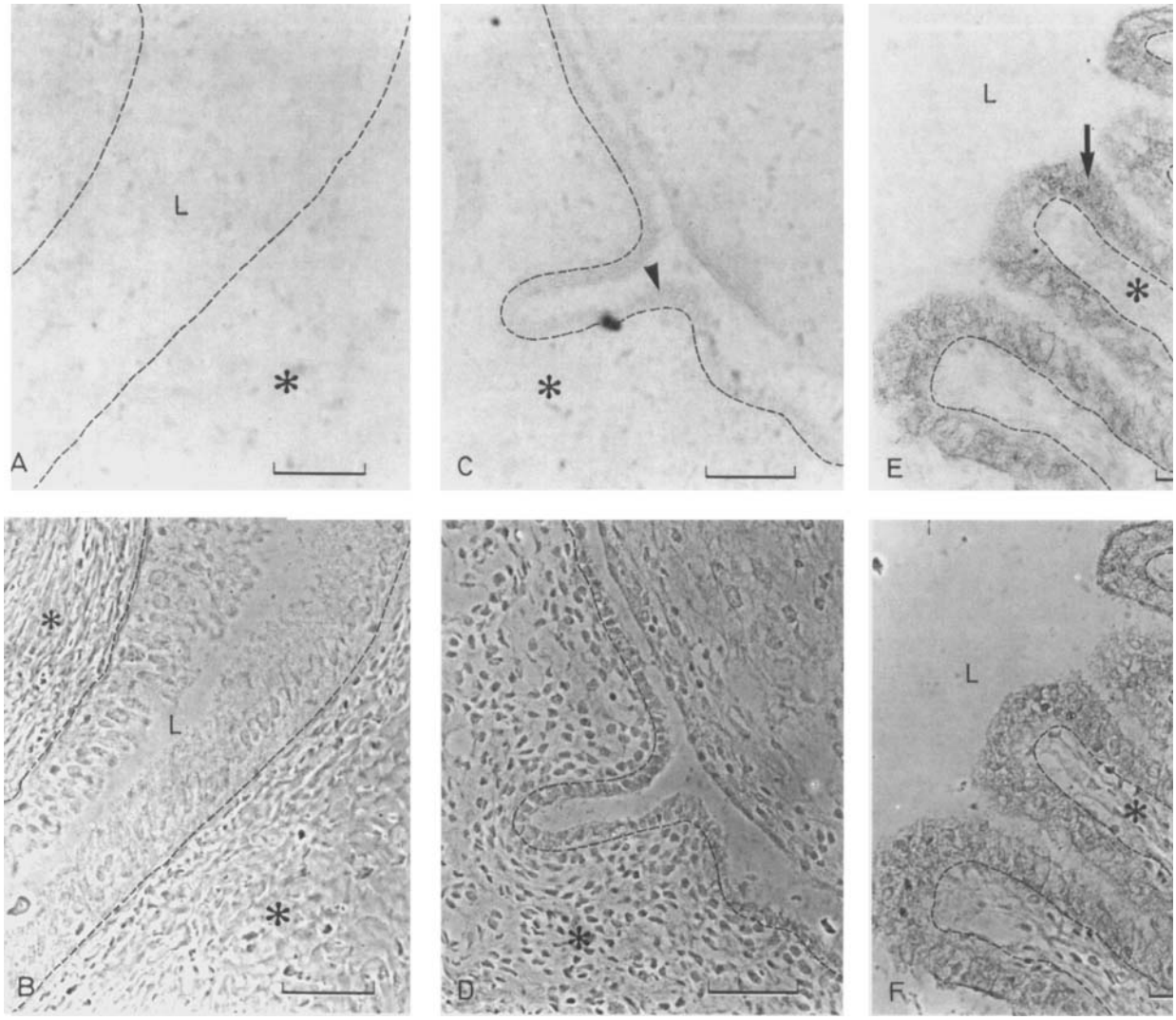

Fig. 5A–F. Distribution of immunoreactivity in rat endometrium; immunogold-silver labeling with monoclonal antibody B.C1 to type-I transglutaminase. **A, C, E** Bright-field microscopy; **B, D, F** phase-contrast microscopy of the same sections. *Dotted lines* indicate the position of the basement membrane; *L*, lumen; *asterisk*, lamina propria. **A, B** Endometrium during estrus; **C, D** endometrium during mid-gestation (15 days); **E, F** endometrium during late gestation (21 days). Immunoreactivity was not detectable in the endometrium of nonpregnant rats (**A**). It was first detectable by immunogold-silver labeling at around 15 days of gestation (**C**, *arrowhead*) and increased in concentration until term. Note the diffuse labeling throughout endometrial cells (**E**, *arrow*). Immunoreactivity decreased after term until it was again no longer detectable. *Bars*, 50 μm

Results

Stratified squamous epithelia

Consistent with our observations in human skin ([48]; Fig. 1A–C), the mouse monoclonal antibody, B.C1, revealed particulate transglutaminase immunoreactivity in the upper spinous, granular, and cornified layers of neonatal rat epidermis. As shown in Fig. 1E, intense staining concentrated at the cell borders was evident in the flattened epidermal cells of the granular layer and, to a lesser extent, in the upper spinous layers. Close inspection revealed faint immunoreactivity in both the cornified and basal layers (Fig. 1D–F) whose level was consistently above that seen in the dermis and the background staining in negative controls. A similar staining pattern was obtained in mature rat skin, although the epidermis was much thinner. Intense staining was also observed in hair follicles, primarily in the outer and inner root sheath cells (Fig. 2). In comparison to control sections, the cortex and medulla of the hair shaft, the sebaceous glands, and the dermis exhibited little or no specific staining.

As shown in Fig. 3, keratinizing epithelia in the oral cavity, esophagus, forestomach, and portions of the stomach exhibited abundant transglutaminase immunoreactivity. As in the epidermis, esophagus (Fig. 3B) and forestomach (Fig. 3C) epithelia showed staining primarily in granular and upper spinous cells. The mucosa of the tongue showed strong staining, with transglutaminase-positive cells extending from the dorsal papillae to the basal layer (Fig. 3A). The stratified mucosa of the stomach (Fig. 3D) exhibited considerable staining throughout the entire living

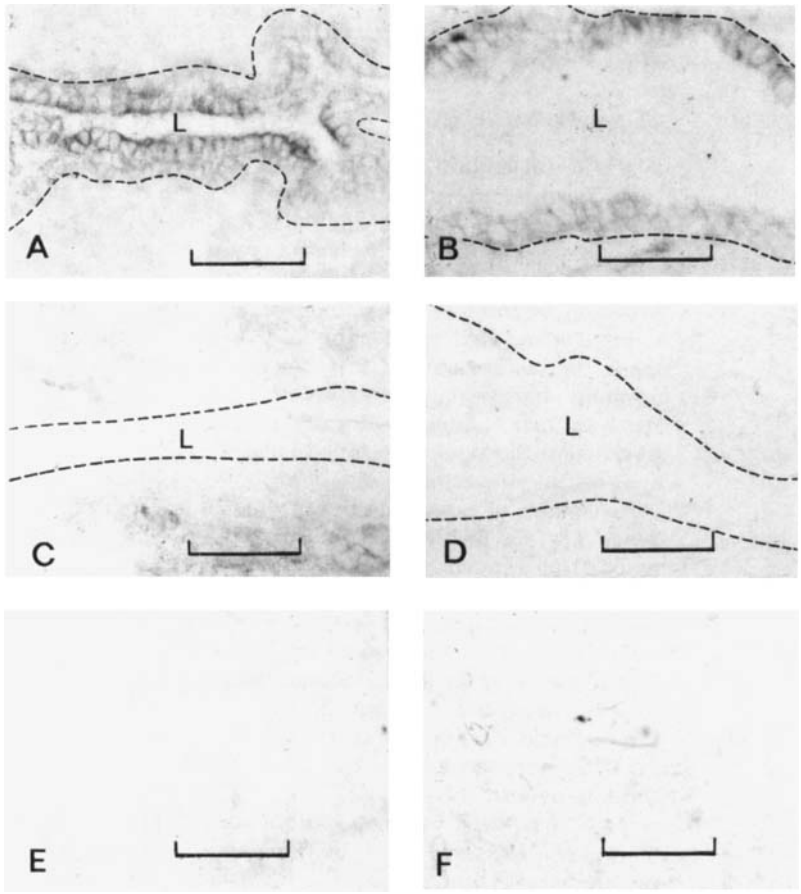

Fig. 6A–F. Immunoreactivity in ductal cells of the mammary and pancreas glands. **A, B, E, F** Immunogold-silver labeling using monoclonal antibody B.C1 against type-1 transglutaminase (bright-field microscopy). *Dotted lines* indicate the position of the basal lamina; *L*, lumen. **A** Ductal cells lining the lactiferous sinus of the mammary gland in an 18-day-pregnant rat; **C** control ascites labeling of the same specimen; **E** mammary gland proper; **B** major pancreatic duct from the same rat; **D** control ascites labeling of the same specimen; **F** pancreas gland proper. *Bars*, 50 μm

layer and faint labeling in the cornified layer. In the columnar and glandular regions of the digestive tract, e.g., the lower portion of the stomach, the small intestine, and the colon, immunoreactivity was undetectable (not shown).

The stratified squamous epithelia of the vagina and cervix were highly immunoreactive. In the cervix during estrus (Fig. 4A), we observed a pattern of strong punctate staining of the granular and upper spinous regions (four to six cell layers in thickness) with little staining in more basal and superficial regions. In contrast, early in pregnancy, the epithelium exhibited rather distinct staining in the basal layer, this gradually increasing in intensity toward the lumen (Fig. 4B). The vaginal and cervical epithelia exhibited parallel morphology and staining patterns in all specimens examined. The staining pattern seen during early pregnancy was maintained throughout pregnancy, even though the epithelia become increasingly glandular and contain an abundance of mucin. During pregnancy, all layers clearly showed immunoreactivity. In the highly mucoid state that is characteristic of late-term pregnancy (Fig. 4D), the staining was most intense in the vicinity of the basal layer, although it was evident throughout the more superficial part of the vaginal epithelium.

Transglutaminase was not detectable in the estrous endometrium (Fig. 5A, B). During pregnancy, positive staining became barely visible at approximately day 15 of gestation (Fig. 5C, D), became easily seen near term (Fig. 5E, F), and gradually declined in intensity during weaning of the young. In the epithelium, which remains a single layer of cuboidal to columnar cells, the staining appeared to be rather evenly distributed throughout the cytoplasm of the immunoreactive cells.

Other epithelia

The major ducts or galactophores in the mammary gland, which are extensions of the nipple epidermis, were always immunopositive. However, the smaller branching lactiferous ducts (Fig. 6A) contained detectable amounts of transglutaminase only during late pregnancy. The major pancreatic ducts, which are negative in males and nonpregnant females, also acquired immunoreactivity at this time (Fig. 6B). The glandular epithelial cells were negative in all cases (Fig. 6E, F). The appearance of transglutaminase in these ducts paralleled its appearance in the endometrium (Fig. 5), suggesting that it is under concerted hormonal control.

Table 1 summarizes in a semiquantitative fashion the observed staining patterns in the tissues examined. In general, with the exceptions noted, immunoreactivity was restricted to stratified squamous epithelia. Thus, immunoreactivity was evident at a low level in the most superficial layer of the corneal epithelium and in the outer epithelial cells of the lens. The kidneys (cortex and medulla), ureters, and transitional epithelium of the proximal half of the urethra were also negative, but the urethral epithelium gradually acquired immunoreactivity as it became more stratified and squamous in the penis (not shown). The immunoreac-

Table 1. Distribution of type-I transglutaminase immunoreactivity in frozen sections of rat tissues[a]

Tissue	Immunoreactivity
Skin	
Epidermis	
Basal layer[b]	+
Spinous layer	+
Granular layer	+ + + +
Cornified layer	±
Hair follicle	
Outer root sheath	+ + +
Inner root sheath	+ + +
Sebaceous glands	−
Dermis	−
Digestive Tract	
Tongue	+ + + +
Esophagus	+ + + +
Forestomach	+ + + +
Stomach	+ + +
Duodenum	−
Jejunum	−
Ileum	−
Colon	−
Respiratory System	
Trachea	−
Bronchioles	−
Lung parenchyma	−
Urinary System	
Kidney	−
Bladder	−
Penile urethra	+ + +
Liver	−
Mesothelium	±
Glands	
Pituitary	−
Thyroid	−
Adrenal	−
Pancreas	−
Prostate[c]	−
Seminal vesicle	−
Submandibular	−
Pancreas:	
gland	−
major duct[d]	+
Mammary:	
gland	−
major ducts[d]	+ +
Genital Tract	
Vagina	+ + + +
Cervix	+ + + +
Endometrium:	
estrous	−
pregnant[d]	+ + +
Eye	
Cornea	+
Lens	±
Retina	−

Degree of reactivity (judged microscopically from the staining intensity) ranging from very strong (+ + + +) to weak (+), marginal (±), or negative (−)

tivity in the mesothelium of the pancreatic capsule was, if present at all, marginal.

Cultured epithelial cells

Work in our laboratory has recently shown that cells cultured from several rat epithelia express type-I particulate transglutaminase which is immunoprecipitable with B.C1 antibody [48] and which displays chromatographic and gel-electrophoretic properties similar to those of the human keratinocyte enzyme (W. O'Callahan, R. Chakravarty, N.L. Parenteau and R.H. Rice, unpublished). Such cultures were highly immunoreactive with the B.C1 mouse monoclonal antibody and displayed two noteworthy features with respect to their staining patterns. First, epithelial cells cultured from the three transglutaminase-negative tissues examined (trachea, seminal vesicle, and urinary bladder) were quite capable of expressing the enzyme, as shown in Fig. 8. When bladder urothelial cells were cultured on gels prepared from rat tail collagen [6], they appeared to form a layer that was two cells thick in transverse sections and were similarly immunopositive (not shown). Second, the immunoreactivity of cells in a given culture was heterogeneous. The intensity of staining was, at best, weakly correlated with cell enlargement or stratification. Indeed, even in colonies initiated by single bladder or tracheal cells that were examined periodically after subculture, a similar nonuniform staining pattern was evident. Thus, the heterogeneity with respect to labeling was not attributable to different types of epithelial cells in the culture population that maintained their individual immunopositive or -negative character.

To examine the transition in transglutaminase expression upon transfer from the tissue to serial culture, we investigated the staining patterns in primary explant outgrowths. Immunoreactivity was evident in all of the cultures, but the distribution of immunopositive epithelial cells differed considerably among the various tissues examined. As illustrated in Fig. 8, the variegated staining pattern displayed in epidermal outgrowths (Fig. 8A, B) was quite similar to that observed in serially passaged epidermal and esophageal epithelial cultures (Fig. 7A, B). (The pattern in esophageal epithelial outgrowths was similar, but with a lower proportion of intensely staining cells.) However, in vaginal outgrowths, the great majority of cells adjacent to the explanted tissue were, at most, marginally immunoreactive, while the cells near the periphery (many of them enlarged and flattened squames) were highly immunopositive (Fig. 8C). Bladder urothelial outgrowths (Fig. 8D) exhibited a zone of weakly reactive or unreactive cells emerging from the tissue, this being surrounded by a zone (that became progressively larger) of more immunopositive cells. In contrast, seminal vesicle outgrowths (Fig. 8E) exhibited strong staining even in small cells adjacent to and newly emerging from the tissue. The variegation in the staining pattern resembled that observed in epidermal outgrowths.

[b] Reactivity of basal layer judged as being marginal or negative in esophageal epithelia, and moderately strong in vaginal and cervical epithelia

[c] All lobes, including the coagulating gland

[d] Maximal reactivity detected only during late pregnancy and early postpartum

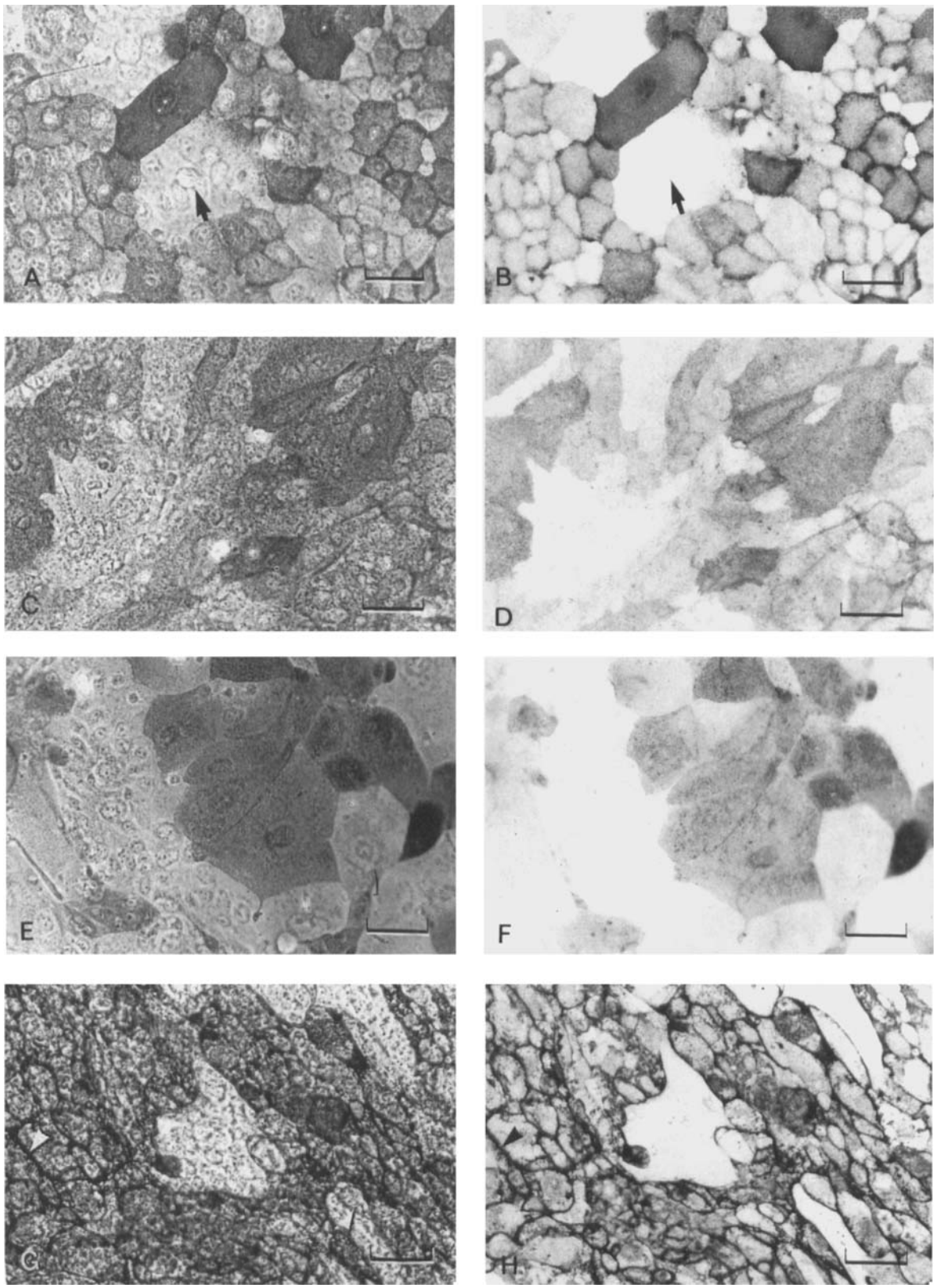

Fig. 7A–H. Immunoreactivity in cultured epithelial cells. Phase-contrast (**A, C, E, G**) and bright-field (**B, D, F, H**) photomicrographs of cells derived from various tissues labeled with B.C1 monoclonal antibody against type-I transglutaminase using immunogold-silver immunocytochemistry. **A, B** Esophageal cells; **C, D** bladder cells; **E, F** tracheal cells; **G, H** seminal vesicle cells. *Arrows* indicate the nucleus of a large squame devoid of enzyme. Note the heterogeneity of the labeling in esophageal cells. The expression of type-I transglutaminase does not appear to be dependent on cell size. Note the peripheral concentration of enzyme (*arrowheads*) and the distinct morphology of the seminal-vesicle-derived epithelium. *Bars*, 50 μm

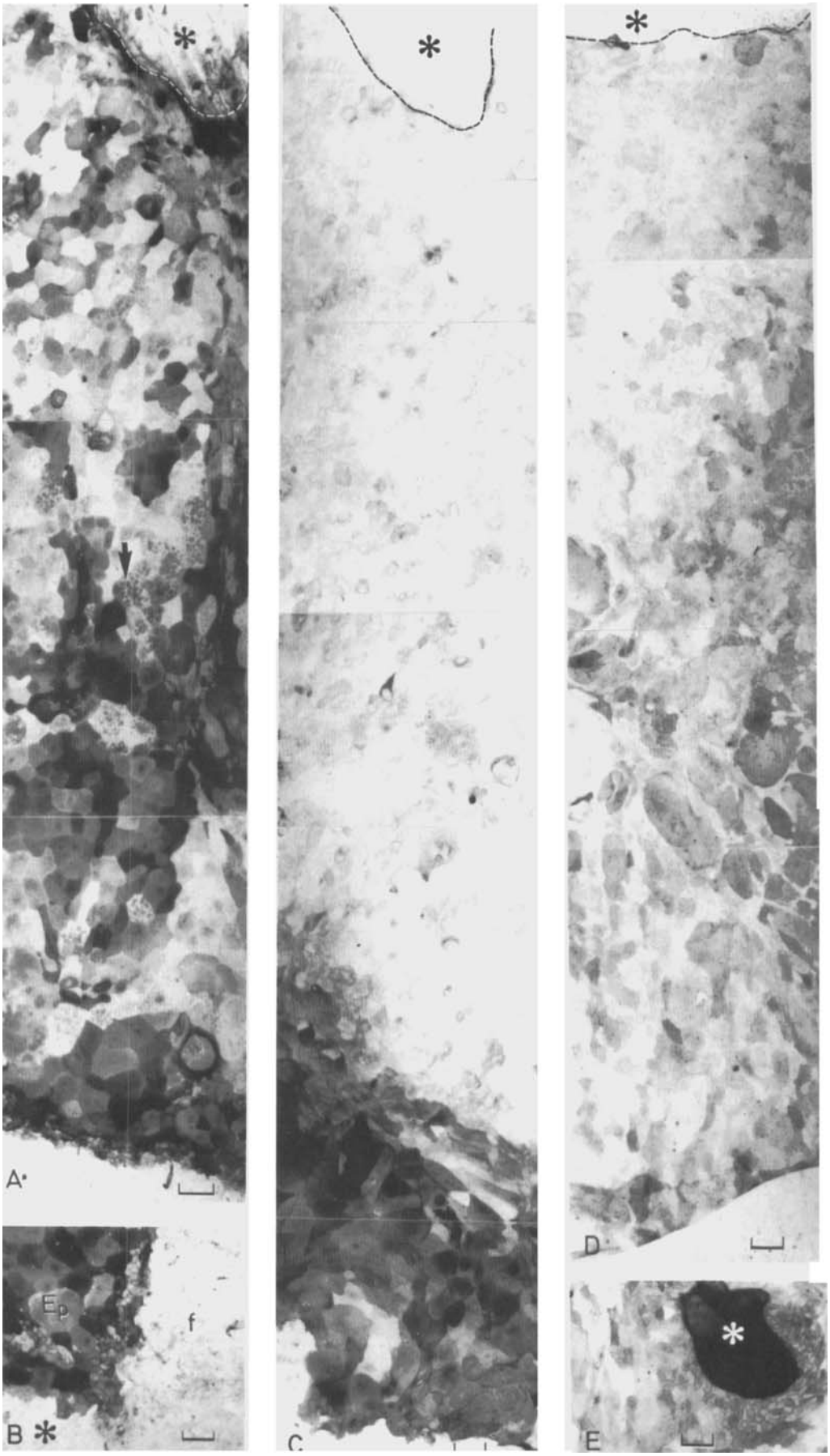

Fig. 8A–E. Distribution of transglutaminase immunoreactivity in epithelial outgrowths from tissue explants. Cultures of epidermal (**A, B**), vaginal (**C**), bladder (**D**), and seminal vesicle (**E**) tissue segments were labeled with B.C1 monoclonal antibody and immunogold-silver. In **B**, note that the epidermal cell outgrowths (Ep) exhibit immunoreactivity, whereas the dermal fibroblasts (*f*) do not. The *asterisks* indicate the positions of tissue segments, which were removed in some cases prior to mounting of the coverslips. *Bars*, 100 μm

Discussion

By immunofluorescence staining of human skin sections, we have previously shown that keratinocyte (type I) transglutaminase is predominantly expressed in the upper stratum spinosum and stratum granulosum [48]. The present results for rats are quite compatible with that finding, but application of the more sensitive silver-enhanced immunogold technique permitted the detection of enzyme expression at low levels even in the stratum basale of rat and human skin. The degree of basal cell expression, however, varied among the stratified squamous epithelia examined, being readily visible in the vagina, for example, but difficult to discern in the esophagus. The generally weak staining seen in the stratum corneum suggests masking or loss of antigenicity by cross-linking or digestive processes characteristic of terminal differentiation.

In rat tissues, type-I transglutaminase immunoreactivity was found predominantly in stratified squamous epithelia, where expression at high levels was characteristic of an intermediate, suprabasal state of differentiation. The low level of expression in the lens, cornea, and mammary galactophores probably reflects the embryological origin of these epithelia from epidermis. In striking contrast to this pattern, however, was the staining observed in the endometrium, the main duct of the pancreas, and the branching lactiferous ducts of the mammary gland. During pregnancy, transglutarminase expression is clearly under different (presumably hormonal) control at these sites, seemingly being independent of any modulation toward a keratinocyte character. The transglutaminase expression observed was also clearly compatible with the highly mucinous state of vaginal epithelial cells late in pregnancy. The influence of hormonal conditions was evident in the lower genital tract due to the degree of staining of basaloid cells and the obvious difference in diffuse versus punctate staining. If not simply due to a higher level of enzyme expression, the latter effect could result from alterations in membrane properties or even, as suggested by the finding of some soluble type-I transglutaminase in cultured keratinocytes [48], from an increase in the cytosolic form.

The keratinocyte transglutaminase studied in the present investigation is distinct from any previously reported with respect to its tissue distribution. We refer especially to the absence of immunoreactivity in the liver, lung, and coagulating gland, in which novel forms of transglutaminase have been described [7, 10, 19, 46, 51]. The relationship of this keratinocyte enzyme to those purified from epidermis and hair follicle remains to be clarified. The B.C1 antibody stains epidermis and hair follicles, while polyclonal antisera raised against rat epidermal and hair-follicle enzymes have been obtained that stain only epidermis or hair follicle, respectively [34]. In view of the proteolytic cleavage to which such enzymes can be subject [31], it is conceivable that differential processing in the epidermis and hair follicle could account for differences in the antigenic determinants exhibited. Alternately, the soluble epidermal enzyme could perhaps be derived from the tissue (type II) transglutaminase, which keratinocytes are capable of expressing, at least in culture [24, 48]. Attempts to show immunochemical relatedness between the hair-follicle and tissue enzymes have been reported to have been unsuccessful [17]. The B.C1 antibody shows no reactivity toward the tissue (type II) transglutaminase by immunoprecipitation [48] or by the immunostaining of human squamous cell carcinoma (SCC-4) cultures treated with retinoic acid (present study), whose cells express only the tissue form [42]. The possibility that at least some transglutaminases are related by exon shuffling [14] bears further examination.

Previous investigations of the properties of cultured rat epithelial cells in the 3T3 feeder layer system have demonstrated convergence toward a keratinocyte phenotype, as judged by high keratin content and the ability to form cross-linked envelopes [35]. The present study shows that, in addition, such cells display substantial levels of keratinocyte (type I) transglutaminase. In certain nonkeratinizing epithelia, notably the mammary gland, it is plausible that transglutaminase-positive cells could be selected by the culture conditions from a pre-existing population present in the tissue (e.g., ductal cells). This interpretation is consistent with the identification in mouse mammary-gland organ cultures of a minority of epithelial cells that are capable of squamous differentiation [44].

Rather than selection, however, the observed transglutaminase expression in explant outgrowths suggests a more complex process of adaptation to culture, which is influenced by the origin of the cultured tissue. Under these circumstances, the various patterns of transglutaminase induction may reflect intrinsic differences in sensitivity to alterations in the microenvironment of germinative cells (e.g., different substratum and medium components, deprivation of stromal influence). With respect to seminal vesicle epithelial cells and bladder urothelial cells, the expression of transglutaminase suggests that, in culture, the cells undergo a process resembling squamous metaplasia, in which a change in gene expression may occur concomitant with growth. Such a change appears to occur rapidly in seminal vesicle outgrowths, in which small cells emerging from the explant already exhibit substantial immunoreactivity. As shown in the bladder explants, the process need not be so prompt. Our results suggest, but do not prove, that cultivation permits the expansion of a major germinative cell population in these nonsquamous epithelia, in which a reprogramming phenomenon is induced. Further investigation of organ cultures by immunohistochemically localizing keratinocyte transglutaminase may permit the identification of specific cells capable of expressing a keratinocyte character.

It remains to be elucidated which intracellular mechanisms mediate the induction of keratinocyte character or squamous metaplasia by physiological and pathological conditions or by specific agents such as cyclic AMP [43], diethylstilbestrol [28], and serum factors, including transforming growth factor β [29]. Keratinocyte (type I) transglutaminase may prove especially valuable as a marker for elucidating these mechanisms. The reversibility of the striking change in expression of the enzyme that occurs in certain epithelial cells upon cultivation is uncertain. However, it is intriguing that changes in properties that occur upon the cultivation of cells from the female genital tract [11] and stratified squamous [15] and tracheal [52] epithelia need not be irreversible, since suitable transplantation into animal hosts can restore lost functions. This phenomenon could plausibly be attributed, at least in part, to the alteration of DNA methylation patterns, which is known to influence differentiation and to occur in cultured cells [23]. In any case, the investigation of transglutaminase expression at the transcriptional level in rat epithelial cells may help to clarify which intracellular factors are responsible

for the induction and maintenance of the synthesis of this enzyme during keratinocyte differentiation and environmental modulation.

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