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Distribution of β1 Integrin Subunit in Rat Seminiferous Epithelium

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ABSTRACT

We have studied the presence and distribution of β1 integrins in the seminiferous epithelium of prepubertal and adult rats. Our immunofluorescence data show that in the adult the antibody recognizes specific areas localized around the heads of elongating and maturing spermatids and above spermatogonia at stages I–VIII. The following were found to be negative: a) areas adjacent to spermatogonia at stages IX–XIV and adjacent to spermatocytes and to round spermatids; b) spermiated spermatozoa. In the prepubertal rat, positive tubules are first apparent around Day 17 of age. Immunofluorescence and immunoprecipitation studies show that Sertoli cell monolayers from 3-wk-old rats express β integrins in vitro.

INTRODUCTION

Integrins, a well characterized family of cell adhesion receptors, are known to mediate cell-substrate and cell-cell interactions [1–3]. These highly conserved transmembrane molecules are heterodimers composed of non-covalently bound α and β chains. So far, seven different β chains have been identified, each of which has been found to form complexes with one of at least 11 different α chains. Since a certain degree of specificity in such association exists, integrin molecules are currently grouped into subfamilies according to their type of β chain [1–3]. Among these, the β1 family is the most represented and is known to be involved not only in the recognition of most intercellular substrates, such as fibronectin [4–7], collagens [8, 9], laminin [10–13], vitronectin [14, 15], tenascin [17], but also in cell-to-cell contacts [2].

It has been known for some time that some epithelial cells express integrins on their basal aspect as well as in areas of cell-to-cell contact. In cultures of stratified keratinocytes [17, 18] the β1 subunit has been reported primarily in areas of cell-cell contacts; in nonstratified cultures of keratinocytes, β1 integrins have been observed to relocate from basal to cell-cell contacts during induced aggregation [19]. On the other hand in intact skin [19], in kidney epithelium [20], and in other pluristratified and monostratified epithelia in a variety of organs [21], β1 integrins are also localized at the site of attachment to the basement membrane. The seminiferous epithelium represents a very particular type of epithelium; in fact it is composed of two completely different cellular components: Sertoli cells—perennial somatic cells forming a columnar monolayer spanning the epithelium from the basis to the lumen, and germ cells—a dynamic population organized in several layers corresponding to sequential differentiating stages. Thus, a single Sertoli cell is simultaneously in contact with the basal lamina, with adjoining Sertoli cells, and with mitotic, meiotic, and post-meiotic germ cells, each of these contacts being confined to a distinct area of the Sertoli cell surface. The seminiferous epithelium represents therefore a particularly interesting model in which the presence of integrin receptors can provide information both on the specificity of integrin-mediated contacts and on the different types of membrane interactions operating during spermatogenic progression.

MATERIALS AND METHODS

Wistar rats killed by CO_2 were used for all experiments. The gonads were excised, cut into 2–8 pieces, and either frozen unfixed in liquid nitrogen in OCT compound (Miles, Diagnostics Division, Elkhart, IN) or used for cell preparations.

Cell Preparations

Sertoli cell-enriched primary cultures were prepared by the conventional technique of Dorrington et al. [22] from testes of 3-wk-old animals and cultured at 32°C in Eagle's minimum essential medium (MEM) without serum. The possible presence of contaminating myoid cells was checked by alkaline phosphatase cytochemistry [23] and found to be negligible (less than 1%). When indicated, the cells were detached in 0.125% trypsin (Difco Laboratories, Detroit, MI), 0.02% EDTA in Hanks' solution and cytocentrifuged in a Cytospin 2 (Shandon Southern Products, Ltd., UK) at 800 rpm for 10 min onto gelatin-coated slides. Myoid cells from 3-wk-old rats were purified from peritubular tissue through Percoll density gradient [24] (Percoll, Pharmacia, Uppsala, Sweden). The cells were cultured at 37°C in MEM without serum. Germ cell suspensions were prepared from adult

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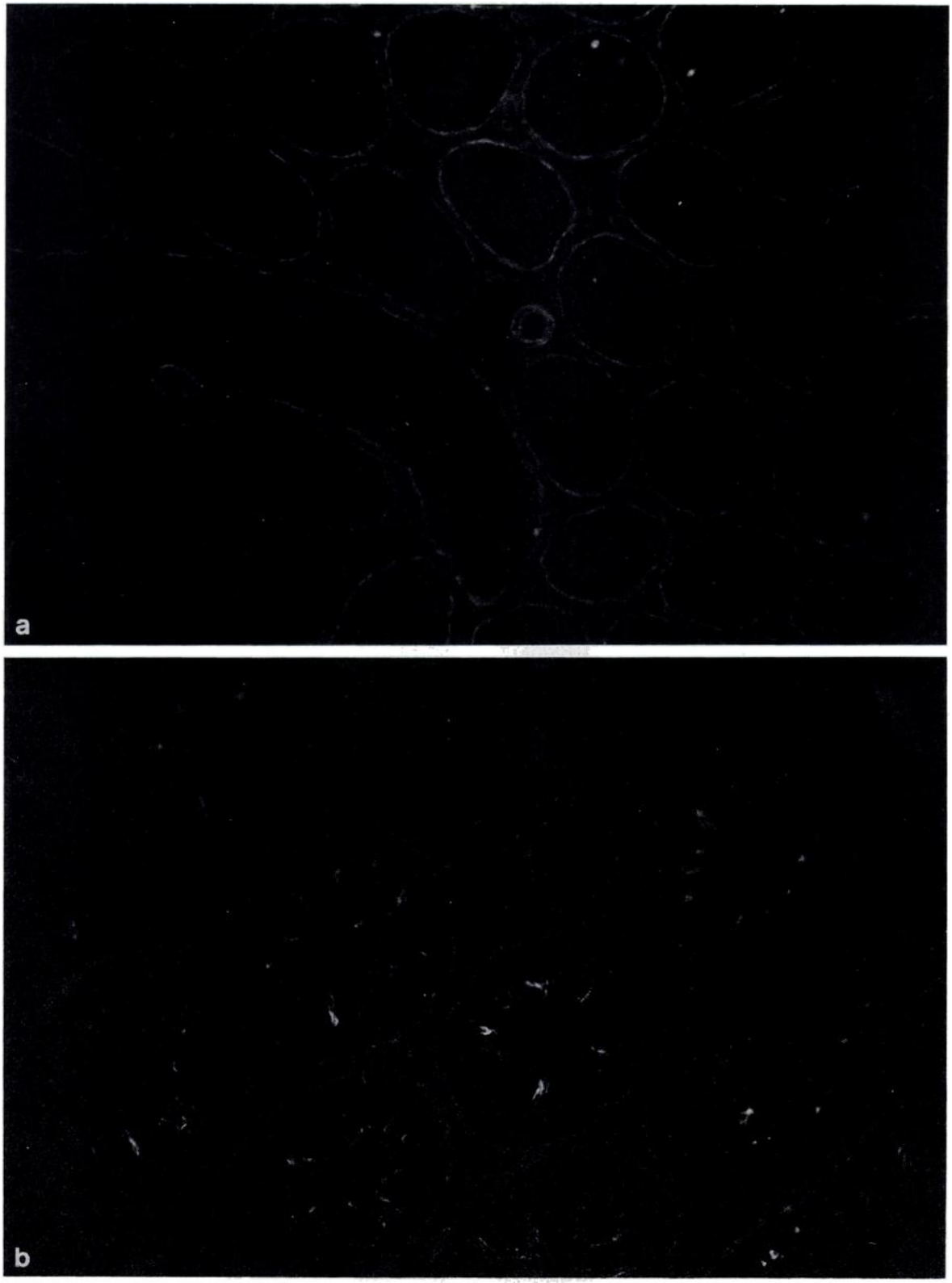


FIG. 1. Cryosections of developing rat testis immunostained for the detection of β1 intgrin subunit. a) At 15 days of age, no fluorescence is apparent within the seminiferous epithelium, whereas the antigen is already expressed in the peritubulum and in vascular muscle cells. ×230. b) at 17 days of age the majority of tubules display restricted fluorescent areas at the level of the seminiferous epithelium. ×370.

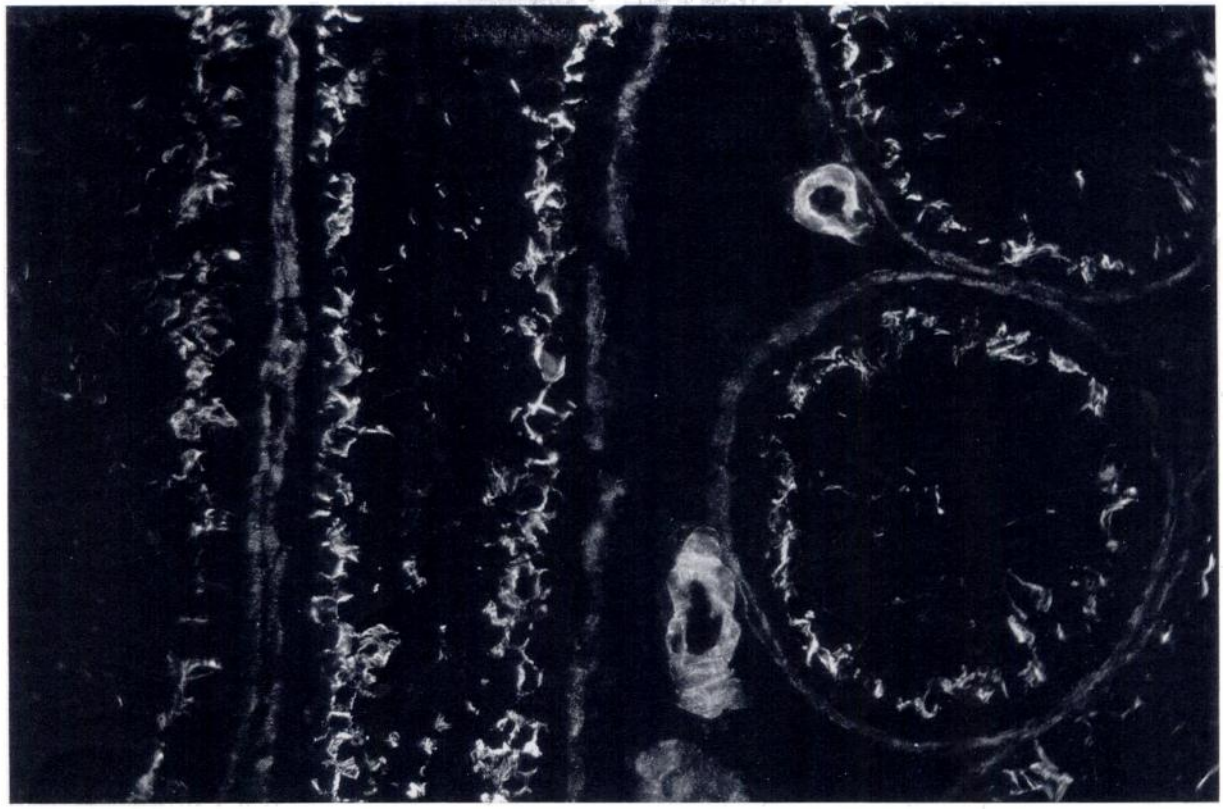


FIG. 2. Cryosection of 20-day-old rat testis immunostained for the detection of β1 integrin subunit. At this developmental age, the antigen is expressed in the seminiferous epithelium of all tubules at a level immediately above the most basal row of cells. ×370.

rats. The testes were decapsulated, the interstitium was enzymatically dispersed in 1 mg/ml collagenase (Serva, Heidelberg, Germany), 2% BSA, 50 μg/ml DNAase (Boehringer, Mannheim, Germany) in MEM for 15 min at room temperature; then the tubules were sedimented and digested again for 45 min at 32°C under constant agitation, mechanically disrupted, and again sedimented for 5 min. The supernatant, composed mainly of isolated germ cells, was cytospun onto gelatin-coated slides as reported above.

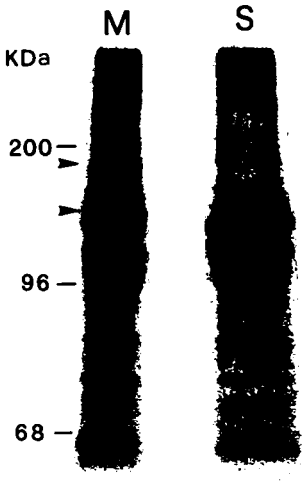


FIG. 3. Identification of β1 integrin subunit from Sertoli cell-enriched cultures (S) and myoid cell cultures (M) from rats aged 3 wk. The cells have been labeled with ^{35}S-methionine for 15 h on the third day of culture. Cell lysis, immunoprecipitation, and SDS-PAGE were performed as described in *Materials and Methods*. Bands of co-precipitated α chains (arrowheads) appear to differ in the two samples.

Immunohistochemistry

Frozen sections (6-μm thick) were cut in a Leitz cryostat (Leitz Wetzlar GMBH, Wetzlar, Germany), air dried for 5 min, and subsequently fixed in acetone-ethanol (1:1 v/v) for 10 min at −20°C. The same fixative was used for cytospun preparations. Before immunostaining, the specimens were treated for 20 min in 2% goat serum to minimize nonspecific antibody binding. Polyclonal antibodies to β1 integrins were prepared as described previously [25] by injecting rabbits with a synthetic peptide derived from the COOH terminal region of the human β1 molecule. The antibodies were purified by affinity chromatography on Sepharose coupled to the synthetic peptide used for immunization, according to a standard procedure. The second antibody used was a fluorescein-conjugated goat anti-rabbit antiserum (Zymed Laboratories Ind., South San Francisco, CA). At the end of the immunostaining procedure, the slides were mounted in 60% glycerol in 0. 1 M Tris buffer, pH 9.3, and viewed in a Leitz Orthoplan fluorescence microscope equipped with phase-contrast optics.

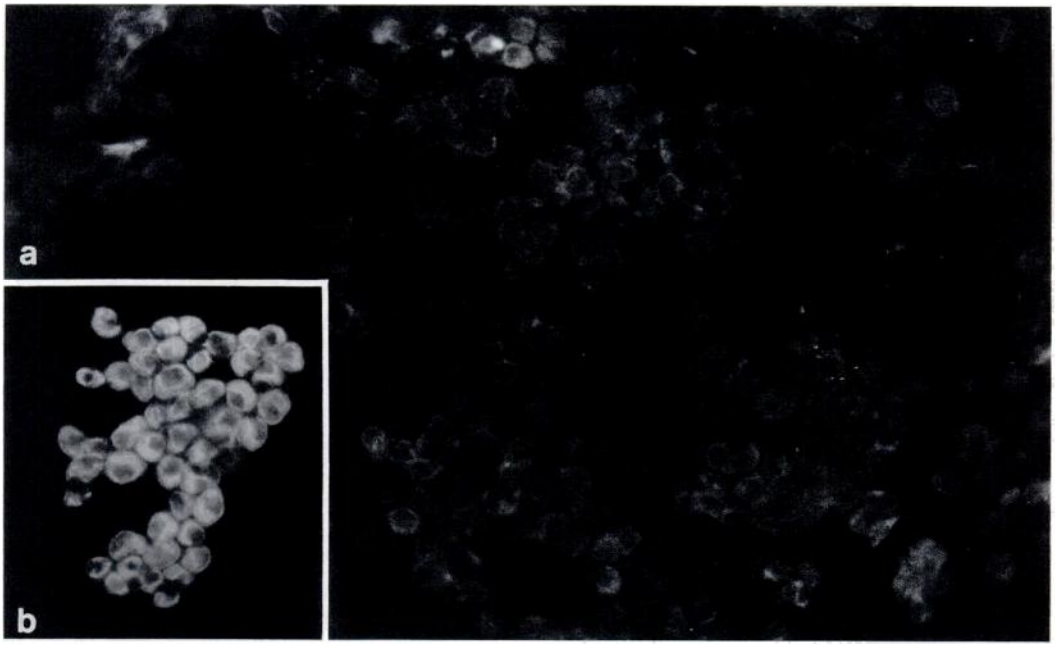


FIG. 4. a) Cytospin preparation of Sertoli cells from 3-wk-old rats after enzymatic detachment from the culture vessel on the third day of culture. The cells were immunostained for β1 integrin subunit. Loss of polarity probably induced a redistribution of the antigen, resulting in a uniform fluorescence of moderate degree. ×370. b) Cytospin preparation of freshly isolated myoid cells immunostained for β1 integrin subunit. The cells appear more brightly fluorescent than in organ cyosections, possibly due to an increase in antigen concentration resulting from the release of tension. ×320.

Immunoprecipitation

Sertoli cell monolayers and myoid cell primary cultures were metabolically labeled with 50 μCi/ml ^{35}S-methionine (Amersham International, Amersham, UK) (spec.act. 800 Ci/mmol) in methionine-free MEM for 15 h on the third day of culture. Integrins were immunoprecipitated using the affinity-purified polyclonal antibodies to the β1 synthetic peptide according to the previously published protocol [25]. Briefly, labeled cells were washed with ice-cold PBS and extracted for 20 min at 4°C with 0.5% Triton X-100 (BDH Chemicals, Essex, UK) in 20 mM Tris-HCl (pH 7.4), 150 mM NaCl (TBS) with 1 mM $CaCl_2$, 1 mM $MgCl_2$, (TBS-Triton buffer), 10 μg/ml leupeptin, 4 μg/ml pepstatin, and 0.1 TIU/ml aprotinin (all from Sigma). After centrifugation at 10 000 × *g* for 10 min, extracts were incubated with the specific antibodies for 1 h at 4°C with gentle agitation. Soluble immunocomplexes were bound to Protein A-Sepharose beads (Pharmacia). After washing, bound material was eluted by boiling beads in 1% SDS (Pierce, Rockford, IL) and analyzed by electrophoresis on 6% acrylamide gels under nonreducing conditions as described [25, 26].

RESULTS

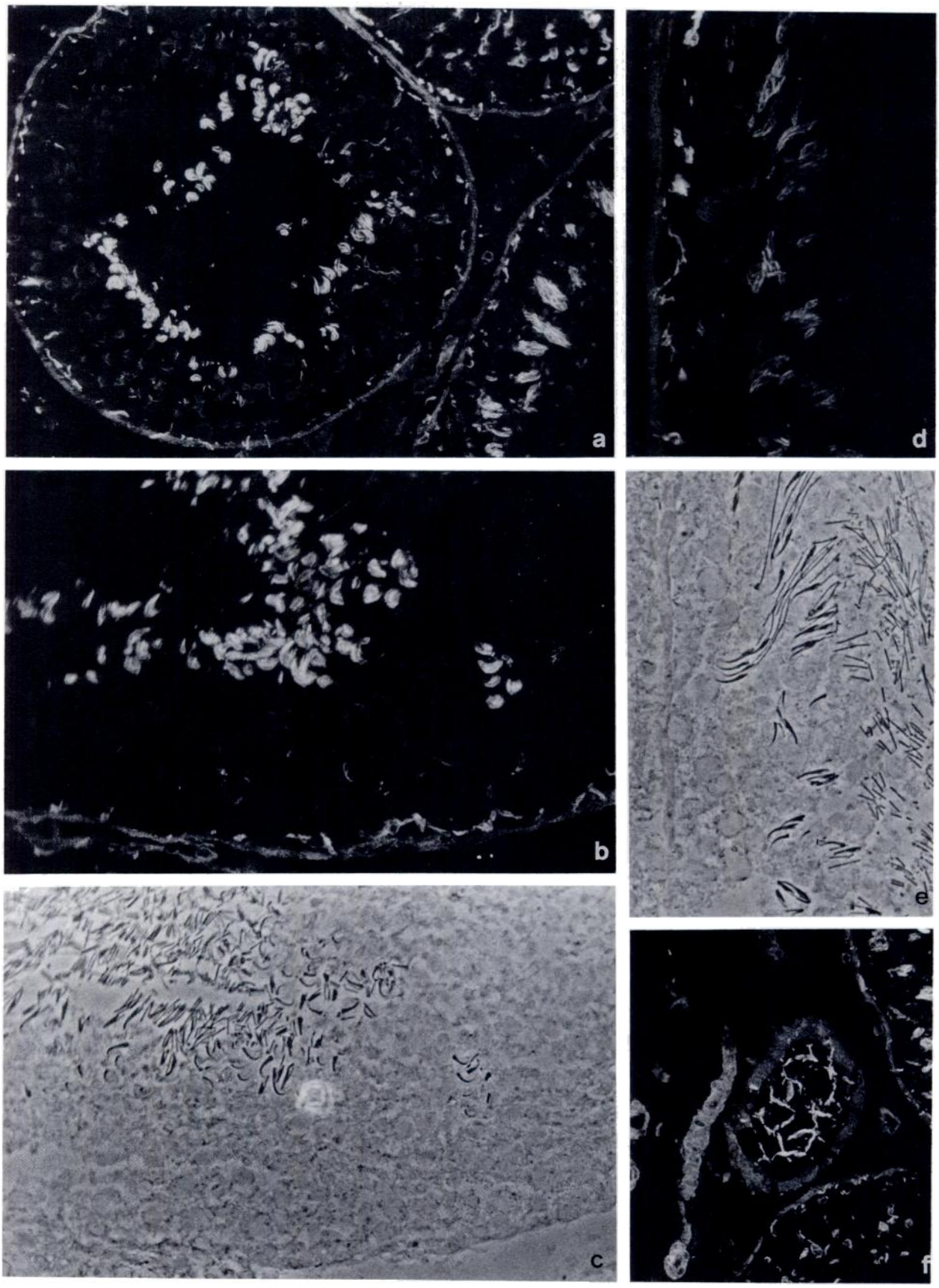
β1 Integrins in Prepubertal Seminiferous Epithelium

Our immunohistochemical findings showed that in the second week of life the only cells expressing β integrins in the seminiferous tubules were peritubular cells, no positivity being present at the level of the seminiferous epithelium (Fig. 1a). Around Day 17, some but not all of the tubular sections displayed a few areas of bright fluorescence (Fig. 1b). In a few days, i.e. at the end of the third week of age, the seminiferous epithelium displayed a regular pattern of positivity in all of the tubular sections. The anti-β1 integrin antibody localized immediately above the most basal cellular row (Fig. 2). Although the fluorescent contour was not easily followed in either cross or longitudinal sections, it appeared to be fairly extended and mainly but not exclusively radially oriented.

Presence of β1 Integrins in Sertoli Cell-Enriched Cultures

Immunoprecipitation of metabolically labeled cell lysates demonstrated that Sertoli cells express β integrins in vitro. Due to the nondenaturing conditions, labeled α chains were coprecipitated (Fig. 3). In parallel myoid cell cultures,

FIG. 5. Cryosections of adult rat testis immunostained for β1 integrin subunit. At the spermatogenetic stages shown, the antigen is expressed at the level of the contacts between Sertoli cells and elongating spermatids and above the basal row of premeiotic cells. Fluorescence around spermatids first appears at the late cap phase (stage VII). a) Central tubule: stage VII; lower right tubule: stage III–IV. ×230. b and c) Stage VI–VII. ×440 (c: phase contrast). d and e) Stage III–IV. ×440 (e: phase contrast). f) Cortically sectioned seminiferous tubule in which the pattern of basal fluorescence is apparent. ×200.



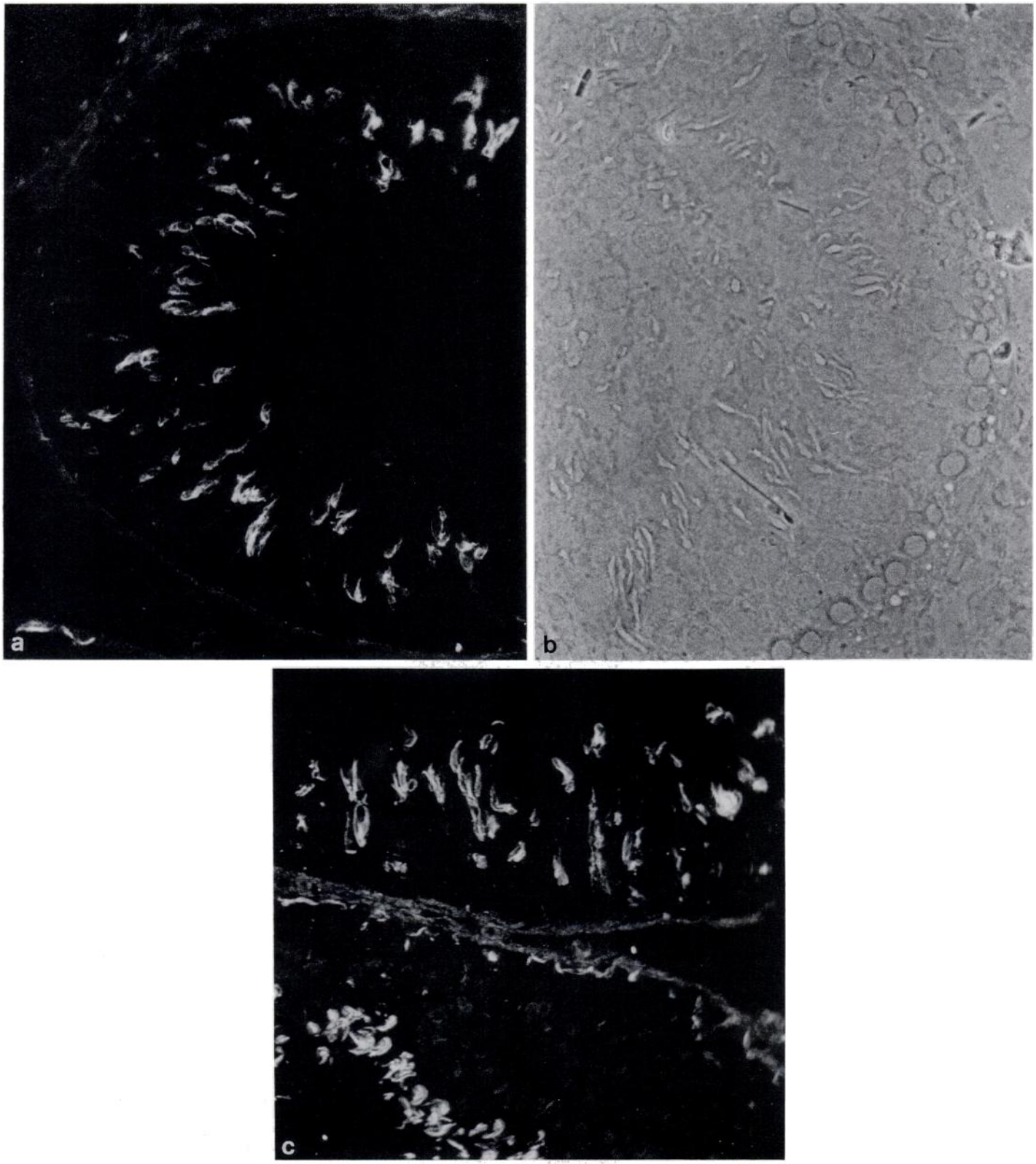


FIG. 6. Cryosections of adult rat testis immunostained for β1 integrin subunit. a) Tubule at stage XI–XIII; the antigen is expressed around the heads of acrosome phase spermatids but not at the level of more basal cells. ×400. b) Tubule at the same stage as in a, shown in phase contrast. ×500. c) Stage dependence of β1 integrin expression is apparent in two adjacent tubules. Lower tubule (stage XI–XIII) displays both basal and adluminal fluorescence. In upper tubule (stage VII), fluorescence is observed only at the level of spermatids. ×370.

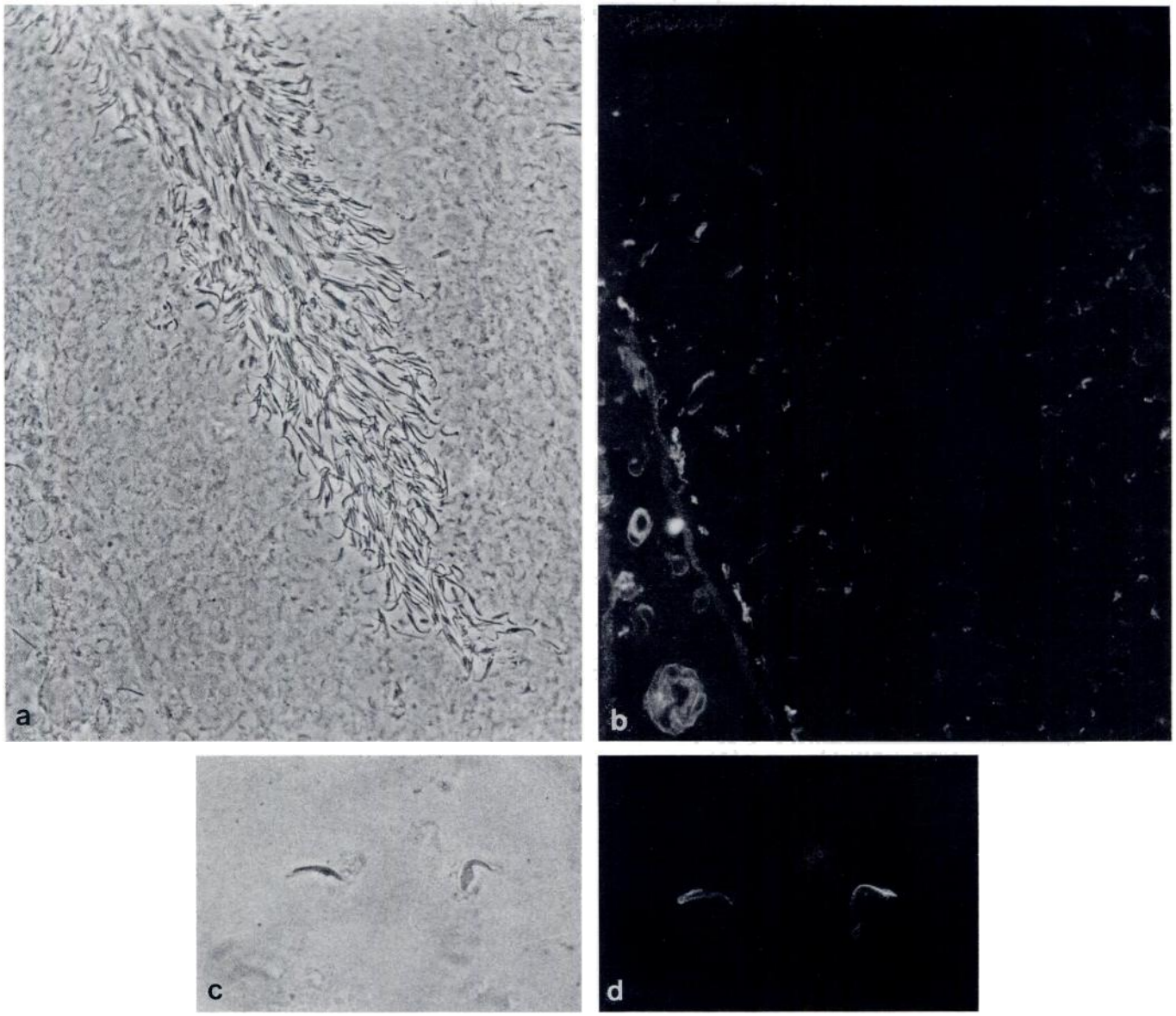


FIG. 7. a and b) Cryosection of seminiferous tubule at stage VIII showing released spermatozoa negative for β1 integrin subunit antibody. ×370 (a: phase contrast). c and d) In cytospun germ cell suspensions from adult rat testes, occasional elongate spermatids (possibly carrying attached ectoplasmic specializations) display rostral fluorescence after β1 integrin subunit immunostaining. ×500 (c: phase contrast).

immunoprecipitation with anti-β1 antibodies confirmed the presence of β integrins in this cell type, in association with α chains differing in molecular mass from those present in Sertoli cells. This difference allowed us to rule out the possibility of significant myoid cell contamination in Sertoli cell cultures. Sertoli cells enzymatically detached at the third day of culture and immunostained after cytocentrifugation displayed a moderate, uniformly distributed fluorescence (Fig. 4a), whereas similarly cytospun freshly isolated myoid cells (Fig. 4b) appeared to be very bright compared both with peritubular fluorescence in cryosectioned tubules and with Sertoli cells. Occasional clusters of negative cells (possibly germ cells) were observed in Sertoli cell-enriched cultures (not shown).

Presence of β1 Integrins at Specific Locations in the Adult Seminiferous Epithelium

The systematic analysis of immunostained tubular sections led to detection of a pattern of β1 integrin distribution constantly specific for stage of the seminiferous epithelium cycle and for cell type. In detail, positive areas were observed at the following locations (Figs. 5–7): a) around the heads of elongating spermatids (late cap phase, stage VII; acrosome phase, stages VIII–XIV; maturation phase, stages I–VII); b) immediately above spermatogonia at stages I–VIII. On the other hand the following were found to be negative for the antibody: a) areas between Sertoli cells or spermatogonia and the basal lamina at any stage; b) areas

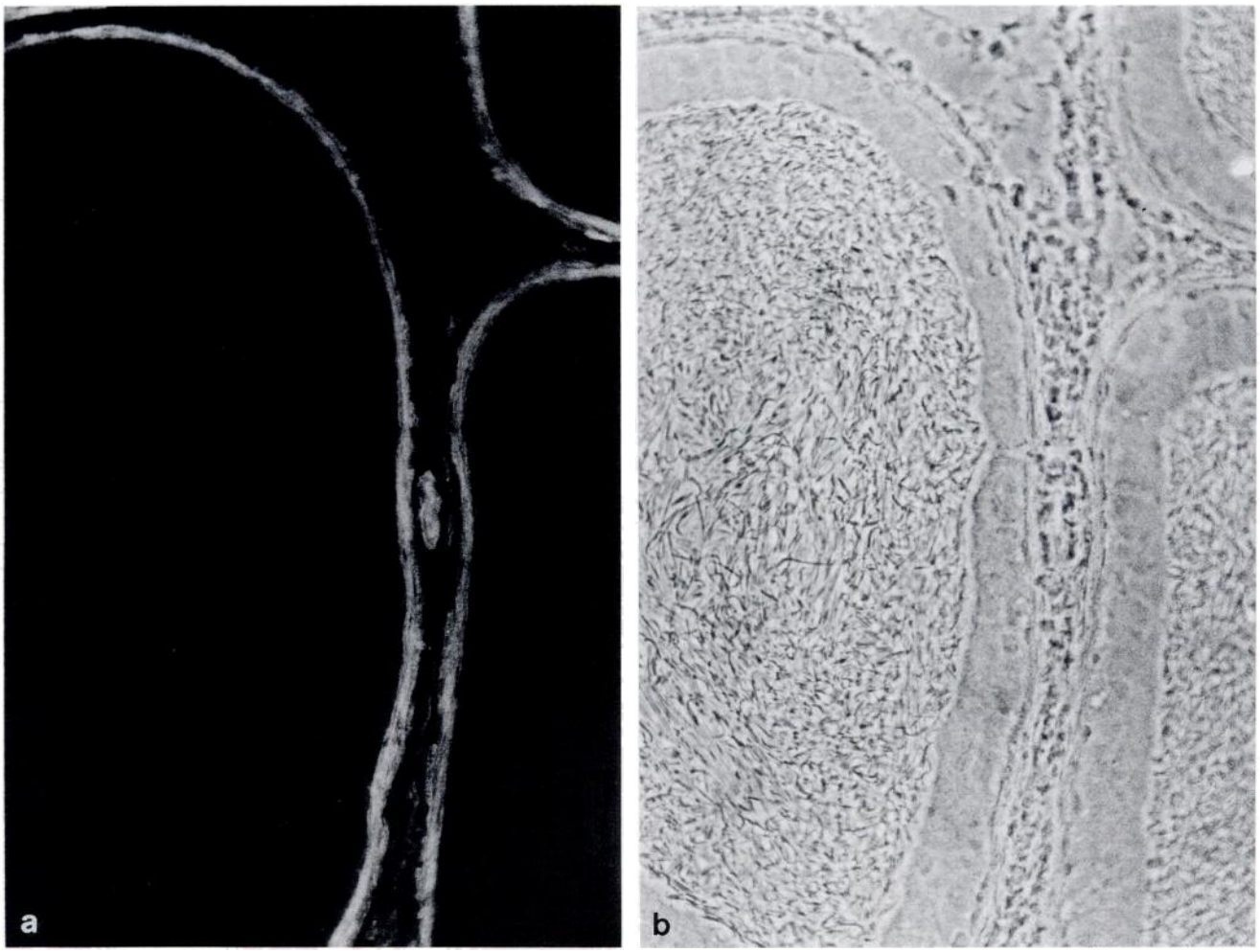


FIG. 8. Cryosection of adult rat epididymis immunostained for β1 integrin subunit. The lumen contains spermatozoa, which appear negative. The tonaca propria and vessels appear fluorescent. ×320 (b: phase contrast).

containing spermatogonia at stages IX-XIV, spermatocytes, or round spermatids (stages I–VI); c) spermiated spermatozoa. Enzymatically isolated spermatids in germ cell suspensions were more often negative (not shown), but occasionally displayed a surface fluorescence in rostral position (Fig. 7). In cryosection, epididymal spermatozoa invariably appeared to be negative (Fig. 8).

DISCUSSION

While differentiating, male germ cells move from the base to the lumen of the seminiferous tubule, along the lateral surface of Sertoli cells. Thus the surface relationship between somatic and germ cells in the seminiferous epithelium is at the same time long lasting and dynamic. From ultrastructural and cytochemical data [27], the nature of such intercellular contacts is known to vary according to the differentiative stage of germ cells and the actual position of the contact along the extended and winding surface of Sertoli cells. Recently, using affinity chromatography, Davis et al. [28] identified laminin-binding proteins of M_r 110 000, 67 000, 55 000, 45 000, 36 000, and 25 000 on the cell membranes of immature rat Sertoli cells; of these, the 67 000, 45 000, and 36 000 M_r proteins were detected by immunofluorescence on the basolateral surface of Sertoli cells—a distribution not incompatible with a role in Sertoli-Sertoli or Sertoli-spermatogonia adhesion. In the same report, anti-β1 antibodies were found to recognize Sertoli cell contours in Sertoli cell monolayers from immature rats and, in cryosections, undefined spots within the epithelium and peritubular cells. On the other hand, immunoblot analysis led the authors to state that Sertoli cells lack detectable amounts of the 140 000 M_r integrin, although they contain lower molecular weight proteins that cross-react with the antibody. In the present report, evidence has been presented for the expression of β1 integrins at the level of definite areas within the seminiferous epithelium (Figs. 5–7). A remarkable feature of the observed localization of the antigen is its stage-specificity, on the basis of which it is tempting to speculate that β1 integrins are components of known junctional

structures. Desmosome-like junctions have been observed between Sertoli cells and germ cells (spermatogonia and spermatocytes) [29]. It seems unlikely that the observed localization of β1 integrins corresponds to that of desmosomes, mainly because the extension of the fluorescent area by far exceeds that of the desmosome-like structures described in the seminiferous epithelium. Moreover, the germ cell types involved are not exactly the same, β1 integrin apparently lacking at the level of spermatocytes. At the level of elongating spermatids, Sertoli cells display specialized cortical structures named ectoplasmic specializations (ESs) [30, 31]. ESs consist of actin-rich subsurface structures, in which vinculin is also present [32], lined on the cytoplasmic aspect by a cistern of endoplasmic reticulum. They are observed both forming a belt at the level of Sertoli-Sertoli junctions, around the cell base, and at the level of the contacts between Sertoli cells and the heads of elongating and maturing spermatids, where they might contribute to the stabilization of membrane domains involved in the adhesion of spermatid heads to Sertoli cells [27]. As described in the present paper, the localization of β1 integrins in the adult rat testis is very similar to that of ectoplasmic specializations at the level of elongating and maturing spermatids. Similarly to ESs, positivity for β1-integrins appears as soon as spermatids start to polarize (stage VII) and seems to follow the outline of the Sertoli cell crypts both in adluminal and basal location, according to the stage of the seminiferous epithelium (Figs. 5–7). Like ESs, positivity for β1 integrin antibody disappears just prior to or at the time of spermiation (Figs. 7–8), a datum in line with the observed negativity of epididymal spermatozoa. The occasional finding of fluorescent spermatid heads is not in contrast with the hypothesis that β1 integrin co-localizes with ESs, since it is known [27] that Sertoli cell membranes at the level of ESs often fail to detach from spermatids when the epithelium is disrupted and germ cells are isolated. As for basal fluorescence, the described stage-specificity is an interesting indication of changes in the molecular composition of cell membranes at the specific sites described during stage-related epithelial remodeling. Ultrastructural immunocytochemical data are needed to define the precise location of β1-integrins in basal position. Two likely possibilities can in fact be envisaged. The antigen may be expressed at the level of inter-Sertoli junctions, perhaps associated with ectoplasmic specializations. This view, which would be in line with that on the distribution of actin observed after NBD-phallacidin staining [27], does not overcome the major difficulty that no stage-specificity has been reported for ectoplasmic specializations in basal position. On the other hand, it cannot be ruled out that the expression of specific molecules such as β1 integrins might indeed be stage-dependent. Alternatively, the antibody may recognize areas between Sertoli cells and certain subclasses of spermatogonia, namely those present at stages II–VIII of the cycle.

In young animals, the antigen starts to be expressed in scattered locations within some of the tubules around Day 17 of age (Fig. 1) and in the totality of the tubules by Day 20; thus the location of the antigen seems more compatible with the possibility of expression at the level of Sertoli-Sertoli contacts. Here again, ultrastructural studies are needed to assess whether the antigen is located within a specific structure and between which cell types: moreover, at this age it is more difficult than it is in the adult to identify the positive cell types. Our data of both immunofluorescence (Fig. 4) and immunoprecipitation (Fig. 3) on cultured Sertoli cells from 3-wk-old animals allow us to state that β1 integrins are expressed by young Sertoli cells, but do not rule out the possibility that the antigen is also present on some subclasses of spermatogonia, while our immunofluorescence evidence suggests that neither spermatocytes nor round spermatids express integrins of the β1 family.

The whole of these data demonstrate that β1 integrin-mediated cellular interactions are present in the seminiferous epithelium from the time a functional permeability barrier [33, 34] develops. Moreover, they demonstrate that the distribution of such molecules in the adult seminiferous epithelium is stage-dependent, suggesting a role in the adhesion of elongating and maturing spermatids to Sertoli cells, but also, at the basal location, in either remodeling of Sertoli-Sertoli interactions or differential adhesivity of different classes of spermatogonia to Sertoli cells.

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