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Unit Gravity Sedimentation Separation of Cells Comprising the Caput Epididymidis of the Rat*

G.J. Killian, Jeanne Snyder**, and R.P. Amann

Dairy Breeding Research Center,
The Pennsylvania State University, Pennsylvania, USA

Summary. Cells from the rat caput epididymidis were separated by unit gravity sedimentation. Purest 12-ml fractions contained 88–95% basal cells or 64–76% principal cells. Ultrastructure of separated cells was similar to that of cells in intact tissue. Viability of separated cells was excellent as determined by dye exclusion tests and cellular ATP content. By combining fractions pools containing $4.0 \pm 0.9 \times 10^6$ cells ($86 \pm 8\%$ basal cells) and $1.4 \pm 0.4 \times 10^6$ cells ($56 \pm 7\%$ principal cells) were obtained. Thus, studies on the function of basal and principal cells from the rat caput epididymidis should be possible.

Key words: Epididymis, rat – Epithelium, isolation – Unit gravity sedimentation.

Introduction

Previously we demonstrated that tissue from hamster caput epididymidis can be enzymatically dispersed and that the resulting mixed cell population can be separated by unit gravity sedimentation into relatively homogeneous populations of epithelial and other cell types (Killian et al., 1976). For physiological studies of epididymal function it is essential to obtain cell populations which are viable, and reasonably pure, and which contain adequate numbers of cells. In preliminary studies with rats, we found that sedimentation of epididymal epithelial cells differed between hamsters and rats. The present study was undertaken to elucidate differences between rat and hamster epididymal cell separations.

Send offprint requests to: G.J. Killian, Department of Biological Sciences, Kent State University, Kent, Ohio 44242, USA

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** Department of Biochemistry, Southwestern Medical School, Dallas, Texas, USA

Materials and Methods

Male Wistar rats (retired breeders from Hilltop, Scottsdale, PA) were maintained on a 12:12 h light: dark cycle. Tissue representing all of region one and the proximal half of region two (Reid and Cleland, 1957) of the caput was taken from both epididymides of one rat, minced and combined with the enzyme dissociation medium 0.75 mg/ml protease, 1 mg/ml collagenase, 1 mg/ml bovine serum albumin [BSA] and 150 μg/ml deoxyribonuclease in Spinner's tissue culture medium; pH 7.2). After 2 h of incubation (Killian et al., 1976), cells were sedimentated (700 g, 10 min) and resuspended in medium-199 containing 1 mg/ml BSA.

Cell separations were performed at unit gravity in a lucite chamber maintained at room temperature. A total of 15–19 × 10^6 somatic cells was used for each separation (Killian et al., 1976). The 48 fractions collected after separation were centrifuged (700 g, 10 min) and the cells were resuspended in 1 ml of medium-199 containing 1 mg/ml BSA. Fractions 25 to 48 were pooled by groups of four before counting cells using a hemocytometer.

Several approaches were used to identify cells in each fraction. In two experiments, isolated cells and intact rat epididymal tissue were prepared for ultrastructural examination (Killian et al., 1976). For three separations, microscopic slides of cells in each fraction were prepared with a Shandon Cytospin (Sewickly, PA) and stained by the Papanicolaou method (PAP) or with oil red 0 (Humason, 1967). Cells were measured and classified according to a scheme developed for hamsters (Killian et al., 1976). To verify the location of fibroblasts in the gradient, cells in separated fractions from two rats were cultured (Killian et al., 1976).

Lymphocytes were identified by immunofluorescence with the use of anti-rat-thymus-lymphocyte serum generated in rabbits or anti-rat-bone-lymphocyte serum (Nutritional Biochemicals). Fluorescein-conjugated goat anti-rabbit gamma globulin (Nutritional Biochemicals) was used in the indirect fluorescent antibody technique. For seven other separations, total and differential cell counts were made on samples representing fractions which had been pooled on the basis of the *anticipated* distribution of the separated cells. The viability of cells was assessed by phase contrast microscopy, vital staining (trypan blue or erythrocin B) and adenine nucleotide content (Killian et al., 1976).

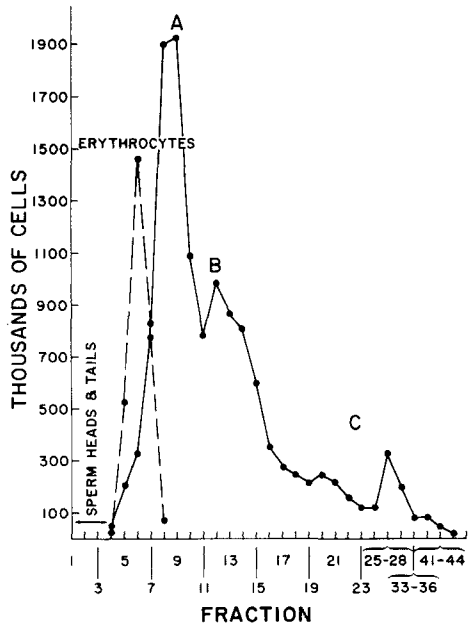

Fig. 1. Cell distribution in fractions after a typical sedimentation of rat caput epididymal cells. The number of cells was determined by duplicate hemacytometer counts. Fractions *1–24* contained 12 ml and were counted singly; fractions *25–48* were counted after pooling into groups of four fractions

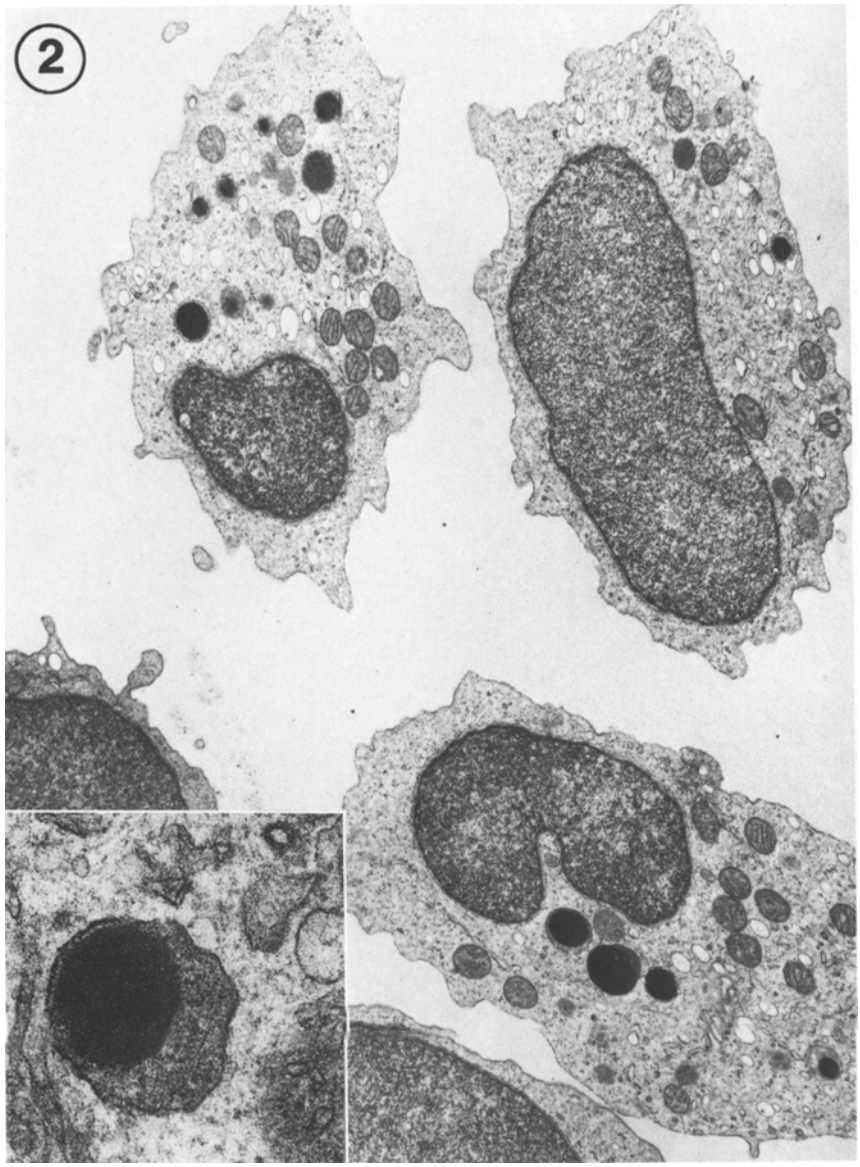

Fig. 2. Electron micrograph of basal cells found in fraction 8. × 10,000. Characterized by homogeneous chromatin and scant cytoplasm where membrane-bounded osmophilic granules were common (insert, × 20,000)

Results

An average of $77 \pm 0.1 \times 10^3$ somatic cells were obtained from dissociations (N = 81). Optimum yields resulted when about 5 ml of enzyme medium was used per 100 mg tissue. During sedimentation, bands of cells became evident after 1.0–1.5 h.

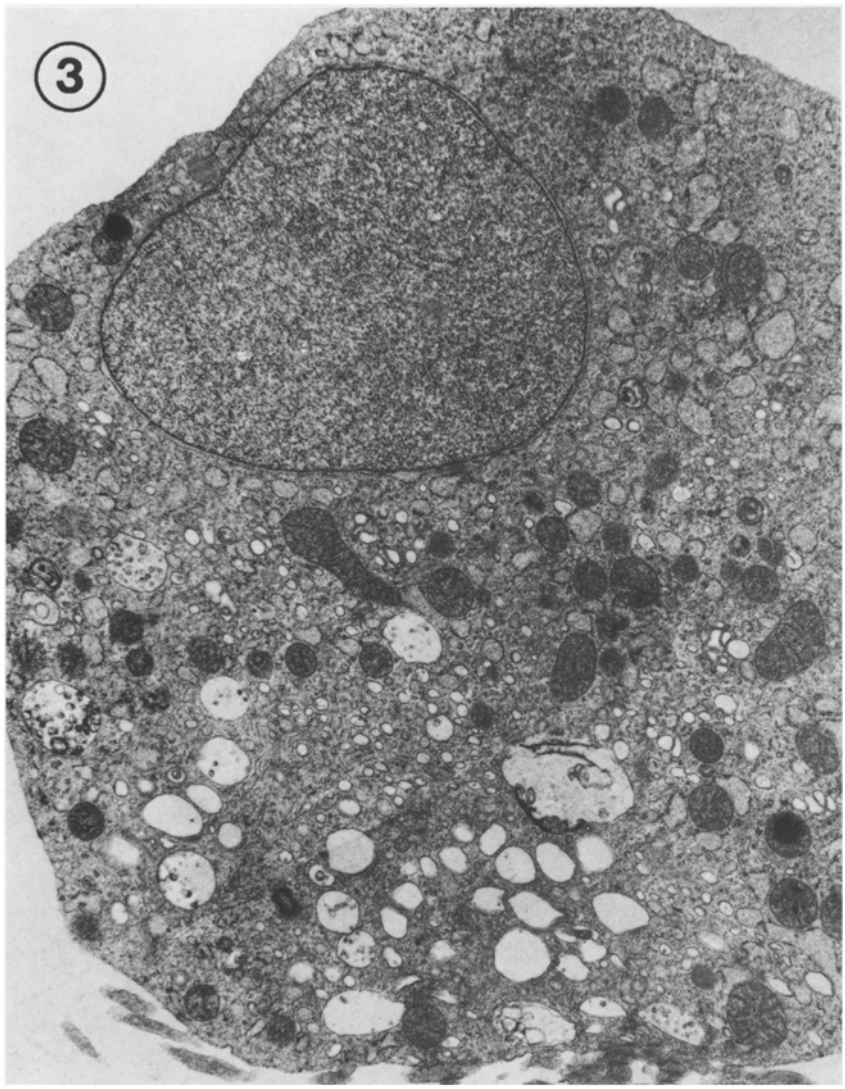

Fig. 3. Principal cell typical of those seen in fractions 19–48. ×10,000

Banding patterns were similar to those observed with hamster cells (Killian et al., 1976), but with rats the band of principal cells was more diffuse. Five of 43 separations were atypical and were discontinued. After sedimentation, $67 \pm 2\%$ of the number of cells loaded into the chamber ($N = 38$) were recovered in five major populations including spermatozoa, erythrocytes and three populations of nucleated somatic cells (Fig. 1). Dead cells accounted for < 5 percent of the cells in any fraction.

The appearance (Fig. 2) of most cells in population A (Fig. 1) was similar to that of basal cells observed in electron micrographs of intact tissue. In some basal

Table 1. Cellular and nuclear diameters for each cell type after sedimentation of dispersed rat caput epididymidis (mean ± S.D.)[a]

Type	Fractions sampled	Diameter (μm)	
		Cell	Nucleus
Erythrocytes	5–6	6.2 ± 0.5	–
Basal cells	8–9	9.6 ± 1.0	8.2 ± 0.9
Lymphocytes or fibroblasts	11–12	11.0 ± 1.0	8.2 ± 1.0
	13–14	17.0 ± 1.8	9.7 ± 0.9
Smooth muscle cells	24–28	10.8 ± 2.2	4.0 ± 1.2
Principal cells	16–17	15.4 ± 2.4	8.0 ± 1.3
	25–28	19.8 ± 3.4	8.2 ± 2.6
	33–36	20.6 ± 2.3	7.6 ± 1.9

[a] Based on eyepiece micrometer measurements of 50 cells of each type in fractions from two sedimentations

cells from both separations examined, large electron-dense, membrane-limited granules were observed (Fig. 2, insert). Similar granules were present in basal cells of intact tissue, although less frequently.

Two cell types predominated in electron micrographs prepared from population B (fractions 12 and 13; not shown). Both types had a lobulated nucleus and scant to moderate cytoplasm. There were fibers in the cytoplasm of some cells. These presumptive fibroblasts were numerous after 3 days in cultures derived from population B, but rare in cultures of populations A or C. Identification of the other cells was less definitive, but they may be lymphocytes.

The predominant cell type in population C was principal cells. They contained a spherical nucleus, abundant rough endoplasmic reticulum and an extensive Golgi apparatus (Fig. 3). Smooth muscle cells of different sizes also were observed in electron micrographs of population C and other fractions. Another cell type seen occasionally in population C had a lobulated nucleus, unusual configurations of smooth endoplasmic reticulum and resembled endothelial cells of intact tissue.

In PAP-stained slides, the general morphology and staining characteristics of each cell type of rat caput epididymal tissue were similar to those described for hamsters (Killian et al., 1976). However, rat smooth muscle cells typically stained red-orange compared to blue-green for hamster smooth muscle cells. Cellular and nuclear sizes (Table 1) aided classification of each cell type in the differential counts (Fig. 4). Erythrocytes were primarily located in gradient fractions 5 and 6. Basal cells were found in fractions 7-11 (Fig. 4) and comprised 91 % of the cells in fractions 8–10. Lymphocytes and fibroblasts were not readily distinguishable from each other in PAP stained slides, but together these cells represented 75 % of the cells in fractions 11–14. A major subpopulation of these cells contained droplets which stained positively for neutral lipid with oil red 0 (Fig. 4). Smooth muscle cells were contained in fractions 17–32 (Fig. 4). Principal cells were concentrated in fractions 16–48 (Fig. 4) with purest fractions averaging 70 %. About 20 % of the principal cells in fractions 16–24 contained numerous droplets of neutral lipid (Fig. 4). A higher

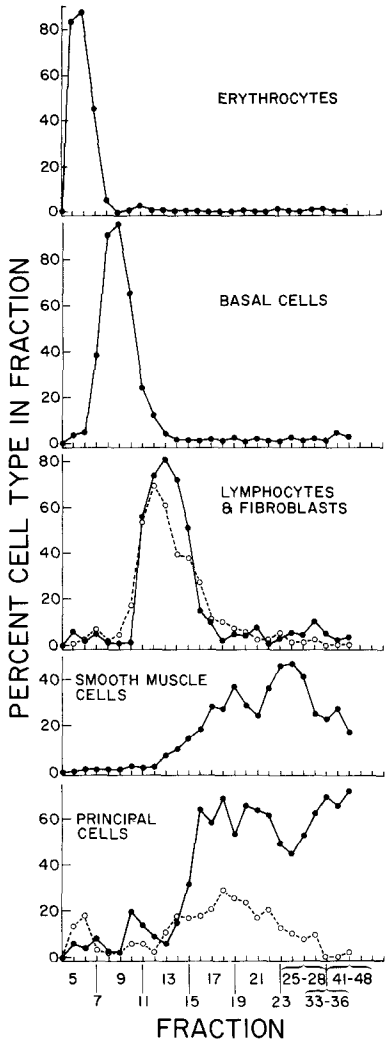

Fig. 4. Distribution of cell types in fractions of the gradient for a typical separation of rat caput epididymal cells. Differential cell counts for this experiment were made on PAP (●———●) and oil red 0 (○ - - - - - ○) stained Cytospin slides (Killian et al., 1976)

percentage of the smaller principal cells in fractions 16–17 contained lipid droplets than that of the larger principal cells in fractions 33–36 (Fig. 4).

The $86 \pm 8\%$ purity of the basal cell fractions 8–9 which were pooled at the time of collection on the basis of the *anticipated* cellular distribution (Table 2) was slightly less than the maximum value in a single fraction. The purity of principal cells, however, was only $56 \pm 7\%$ after pooling.

Cells in fractions grouped to represent the major cell types always contained more ATP than ADP. Basal and principal cells contained 24 ± 3 and 59 ± 6 nM/10^6 cells of ATP plus ADP. In basal cells ATP represented $92 \pm 1\%$ of the total and in principal cells ATP was $101 \pm 5\%$. Thus, the cells were metabolically active.

Table 2. Cell yield and purity in pooled fractions (mean ±S.D.)[a]

Fractions pooled	Total number of cells (10^6)	Percentage of cell type				
		Erythrocytes	Basal cells	Lymphocytes and fibroblasts	Smooth muscle cells	Principal cells
5–6	1.8±0.1	**88±6**	6±4	<1±<1	<1±<1	4±2
8–9	4.0±0.9	2±2	**86±8**	6±4	1±<1	5±3
11–12	1.6±0.4	<1±<1	24±18	65±15	3±2	7±4
13–14	1.0±0.3	<1±<1	4±2	**83±11**	4±2	8±2
15–18	1.0±0.4	<1±<1	2±1	47±10	16±9	34±10
19–48	1.4±0.4	2±1	2±1	17±3	**22±5**	**56±7**
Dissociated[b]	—	18±2	20±1	20±4	12±5	28±6
Maximum enrichment		4.8	4.3	—	1.8	2.0

[a] Mean for 7 sedimentations. Bold faced values designate location of purest fractions for each cell type

[b] Mean for 28 dissociations

Discussion

Rat basal cells recovered after sedimentation were slightly smaller than those from hamsters (Killian et al., 1976), but generally contained a greater number of cytoplasmic organelles. Although membrane-limited granules, resembling lipid, were seen in basal cells (Fig. 2), they did not stain for neutral lipid with oil red 0 (Fig. 4). Similar granules have been observed in intraepithelial lymphocytes in the epididymides of rats, monkeys and hamsters (Hoffer et al., 1973; Dym and Romrell, 1975; Killian et al., 1976), but their chemical nature is unknown. Brandes (1974) compared them to secretory granules of certain endocrine tissues. Since more dense granules were observed in isolated cells than in basal cells in intact tissue, some granules may form as a result of the changing environment. However, similar dense granules were not observed in hamster basal cells isolated by the same method (Killian et al., 1976).

Identification of separated cells in population B (Fig. 1), was difficult. However, based on fine structure and culture studies, it was evident that many of them were fibroblasts. Other cells in population B resembled intraepithelial lymphocytes (Hoffer et al., 1973; Dym and Romrell, 1975). Furthermore, freshly prepared cells from fractions 11 and 12 gave positive immunofluorescent reactions with anti-rat-thymus-lymphocyte serum. Slides reacted with anti-rat-bone-lymphocyte serum and control γ-globulin were negative. The distribution of cells apparently containing rat-thymus-lymphocyte antigen was similar to that of cells containing neutral lipid (Fig. 4).

The principal cell in Figure 3 is typical of most cells seen in fractions 16–48 and similar to the predominant cell type in the epithelium of the rat caput epididymidis. Unlike separated hamster principal cells (Killian et al., 1976), "large" and "small"

principal cells were not clearly distinguishable in differential counts and were represented collectively in Figure 4. The heterogeneity of principal cells in population C (Fig. 1) may represent the presence of functionally different phases of the same cell type although it is possible that two different types of principal cells are present in the caput epididymidis.

The purity of single fractions reached 91 % for basal cells and 70 % for principal cells, but the number of cells recovered in a single fraction ($1–2 \times 10^6$ basal cells or $1–3 \times 10^5$ principal cells) might be insufficient for physiological studies. However, data on glucose utilization, oxygen consumption and carbon dioxide production can be obtained using only 0.5×10^6 dissociated somatic cells (Killian, unpublished). Thus, similar studies based on the basal cells in a single 12-ml fraction should be possible.

Use of a single fraction containing 70 % principal cells would restrict the experiment to $< 3 \times 10^5$ cells and impose severe restraints on experimental design. To obtain sufficient principal cells for study, fractions must be pooled at the expense of purity (Table 2). Use of pooled fractions containing approximately 56 % principal cells would make interpretation of most studies difficult even though the percentage of principal cells would be twice that of any one contaminating cell type (Table 2) and sperm would be absent. Pooling only those fractions with ≥ 60 % principal cells would increase cell numbers to $0.5–1.0 \times 10^6$ cells. Since it is impossible to predict which fractions would contain ≥ 60 % principal cells, pooling would have to be done after differential cell counts on each fraction.

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