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Distribution and Levels of Cellular Retinol- and Cellular Retinoic Acid-Binding Protein in Various Types of Rat Testis Cells[1]

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ABSTRACT

The distribution and levels of cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) were measured in rat testicular peritubular and Sertoli cells and in isolated rat pachytene spermatocytes and spermatids. Two Sertoli cell preparations, one containing some germ cells and another that had been osmotically shocked to destroy germ cells, were examined. CRBP and CRABP levels were measured by specific and sensitive radioimmunoassays. Testicular peritubular cell cytosol preparations were found to contain high levels of CRBP (1.48 ± 0.87 μg CRBP/mg protein) but CRABP could not be detected. The mean CRBP level in Sertoli cell preparations that contained some germ cells was 0.93 ± 0.24 μg CRBP/mg protein; this value was similar to the level of 1.11 ± 0.20 μg CRBP/mg protein measured for Sertoli cells free of germ cells. The level of CRABP found in Sertoli cell preparations containing germ cells (0.81 ± 0.32 μg CRABP/mg protein) was approximately five times greater than was observed in Sertoli cells free of germ cells (0.16 ± 0.03 μg CRABP/mg protein). CRBP and CRABP levels in cultured Sertoli cells were not affected by time in culture for up to five days of culture. Pachytene spermatocytes and spermatids were very enriched in CRABP (0.72 ± 0.26 μg CRABP/mg protein for spermatocytes and 0.65 ± 0.21 μg CRABP/ml protein for spermatids). A search for a high molecular weight retinol-binding protein did not demonstrate the existence of such a protein in Sertoli cell-conditioned medium. In summary, these studies provide quantitative information about the distribution of the cellular retinoid-binding proteins in the cell types that compose the rat testis. The data delineate the high enrichment of CRBP in Sertoli cells and that of CRABP in germ cells.

INTRODUCTION

The role of retinoids in mammalian spermatogenesis has long been recognized. Wolbach and Howe (1925) reported the failure of spermatogenesis in animals deprived of retinol. Later studies (Thomson et al., 1964; Krueger et al., 1974; Huang and Hembree, 1979) have shown that in retinol deficiency, spermatogenesis does not progress beyond early meiosis and is accompanied by extensive degeneration of the germinal epithelium. Other workers (Haneji et al., 1982, 1984), using cultured testis fragments from cryptorchid mice, have reported that retinoic acid may be important in the differentiation of spermatogonia. These workers reported that both retinol and retinoic acid interacts with follicle-stimulating hormone (FSH) to exert effects on spermatogenesis. In vivo, retinoic acid cannot substitute for retinol in the maintenance of normal spermatogenesis (Howell et al., 1963; Huang and Hembree, 1979).

Retinol is transported in plasma bound to a specific transport protein, retinol-binding protein (RBP) (Goodman, 1984). RBP delivers retinol to target tissues for retinol action by a process that may involve specific cell surface receptors for RBP (Rask and Peterson, 1976; Chen and Heller, 1977). Within target cells, retinol is bound to a cytosolic retinol-binding protein (CRBP), which is distinct from

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plasma RBP (Chytil and Ong, 1984). CRBP and the cellular retinoic acid-binding protein (CRABP), which specifically binds retinoic acid within cells, may possibly play roles in the expression of the biological activity of their respective retinoid ligands within cells (Chytil and Ong, 1984).

The cellular localizations of the different retinoid-binding proteins in the rat testis have been examined in several reports (McGuire et al., 1981; Kato et al., 1985a; Porter et al., 1985). Immunohistochemical (Kato et al., 1985a) and autoradiographic (McGuire et al., 1981) studies have localized RBP to the interstitial space of the testis. CRBP has been found to be strikingly localized within the Sertoli cells (Kato et al., 1985a; Porter et al., 1985). In contrast, CRABP was found, on immunohistochemical study, particularly localized in the germ cells (Porter et al., 1985). In addition, a novel, high molecular weight retinol-binding protein was reported by Carson et al. (1984) to be secreted by the murine Sertoli-derived TM-4 cell line. It has been postulated by these authors that this novel binding protein is involved in the transport of retinol either to the lumen of the seminiferous tubules or to the developing germ cells.

We now report the levels of CRBP and CRABP in cultured peritubular and Sertoli cells and in isolated pachytene spermatocytes and spermatids from rat testis. CRBP and CRABP levels were measured by sensitive and specific radioimmunoassays for each protein. Conditioned media from Sertoli cells were examined for the possible presence of the retinol-binding protein reported by Carson et al. (1984) to be secreted by Sertoli-derived cells. These studies extend the available knowledge concerning retinoid metabolism in the testis.

MATERIALS AND METHODS

Preparation and Culture of Rat Testis Cells

Small explants of seminiferous epithelium from 20- to 22-day-old male Wistar rats were made by using the procedures described by Dorrington and Fritz (1975). Testicular fragments were digested for 30 min at 32°C in Hanks' buffer containing 0.25% trypsin (Difco, Detroit, MI) to detach the interstitium. At the end of the incubation, 2% bovine serum albumin was added to inhibit the enzymatic activity, and the tubules were washed twice with Hanks' buffer. The seminiferous tubules were then treated with 1 mg/ml collagenase (Boehringer, Grade II, Mannheim, FRG) for 30 min at 32°C, washed twice with Hanks' buffer, dispersed into small fragments by gentle pipetting, and centrifuged in a graduated conical tube for 2 min at 75 × *g*. The pellet, enriched in Sertoli cells, was resuspended in Minimum Essential Medium (MEM) (Gibco, Paisley, Scotland) at a ratio of 0.1 ml of pelleted material to 1 ml of medium. Cells were incubated at 32°C in a controlled atmosphere at 95% air and 5% CO_2. After three days, some of these Sertoli cell-enriched cultures were subjected to an osmotic shock treatment that destroys germ cells associated with the Sertoli cells but leaves the Sertoli cells unharmed (Galdiere et al., 1981). The osmotic shock treatment was conducted exactly as described previously (Galdieri et al., 1981). Briefly, the 2-day-old Sertoli cells containing germ cells were treated for 2.5 min with a hypotonic solution (20 mM Tris-HCl, pH 7.4) that selectively destroys germ cells without affecting Sertoli cells. The Sertoli cells thus obtained have morphologic and biochemical characteristics indistinguishable from those of untreated cells (Galdieri et al., 1981). Cultures of osmotically treated Sertoli cells were allowed to remain in culture for at least 24 h after this treatment before assay for CRBP and CRABP. Thus, two distinct preparations of Sertoli cells were obtained; one preparation consisted of Sertoli cells free of germ cells, and the second preparation consisted of cells enriched in Sertoli cells but still containing germ cells.

Peritubular cells were isolated according to procedures employed by others in studies of peritubular cell structure and function (Wilson and Griswold, 1979; Hutson and Stocco, 1981; Wright et al., 1981; Skinner and Griswold, 1982). After the enzymatic treatment, as described above, the seminiferous tubules were washed with phosphate-buffered saline (PBS) and allowed to sediment by gravity. The supernatant was removed, and the sedimented tubules were rewashed with PBS and allowed to resediment. The resulting supernatant was removed and pooled with the first; the combined supernatant was centrifuged for 2 min at 50 × *g*. The supernatant was then centrifuged for 5 min at 300 × *g*. The pelleted cells so obtained comprised the peritubular cells used in this work. These cells were resuspended in MEM supplemented with 10% fetal calf serum and cultured at 37°C in a humidified atmosphere at 95% air and 5% CO_2. Within 2 or 3 days, a cellular monolayer was formed. After complete monolayer formation, the cells were washed several times with serum-free

medium and cultured for 24 h in serum-free medium before they were harvested for assay of CRBP and CRABP. Medium obtained from monolayer cultures of these cells did not contain androgen-binding protein. The morphological appearance of the monolayer was characteristic of fibroblasts and was clearly different from the appearance of the Sertoli cell monolayers.

Germ cells were prepared from seminiferous tubules of 34- to 36-day-old male Wistar rats by employing a standard procedure for germ cell isolation (Lam et al., 1970; Adamo et al., 1980). The interstitial tissue of the tubules was removed by treatment with collagenase (1 mg/ml) (Boehringer, Grade II, Manneheim, FRG) in MEM containing 1 mM sodium pyrivate and 2 mM sodium lactate for 15 min at 32°C. The tubules thus obtained were washed twice in PBS and incubated for 30 min with collagenase in MEM containing 1 mg/ml bovine serum albumin, 1 mM sodium pyruvate and 2 mM sodium lactate. The resulting suspension of germ cells was separated by velocity sedimentation at unit gravity in an albumin gradient (Lam et al., 1970; Adamo et al., 1980). Separate fractions containing essentially mid-late pachytene spermatocytes (purity 80–85% as determined by phase-contrast microscopy) and round spermatids (purity 80–95% as determined by phase-contrast microscopy) were collected; the two germ cell-containing fractions were concentrated by centrifugation at 250 × *g* for 20 min, and washed twice with MEM before assay. Each individual germ cell fraction (spermatocytes or spermatids) assayed for CRBP and CRABP was greater than 80% pure.

Shipment and Storage of Cell Preparations

The different testis cell preparations were sent by air express on dry ice from Rome to New York. Cell preparations were shipped as cell pellets or in a small volume of culture medium. For some Sertoli cell preparations, the conditioned media from the Sertoli cell cultures also were sent to New York for analysis. All samples arrived in New York in the frozen state, and were stored at −20°C prior to assay. The samples were assayed within 2 wk after arrival. All cell preparations and some conditioned media from Sertoli cells were assayed for CRBP and CRABP: Sertoli cell-conditioned media were examined for retinol binding activity and some Sertoli cell preparations and media were examined for the presence of plasma RBP.

Assays for CRBP and CRABP

CRBP and CRABP levels were measured by specific and sensitive radioimmunoassays as described in detail previously (Kato et al., 1985a,b). No immunological cross-reactivity between CRBP and CRABP was observed in either radioimmunoassay. Cell samples were thawed and suspended in 2 ml of 1.0% Triton X-100 in 50 mM imidazole, pH 7.4, containing 0.03% bovine serum albumin, 0.01% leupeptin, 0.1% Thimerosal, and 0.15 M sodium chloride. Samples were homogenized by using a Polytron homogenizer for 1 min at setting 6 and centrifuged at 100,000 × *g* for 1 h. After centrifugation, the supernatants were removed and immediately assayed for CRBP and CRABP. The radioimmunoassays for CRBP and CRABP employed identical protocols and were carried out as described elsewhere (Kato et al., 1985a,b). The assays were carried out in the homogenization buffer, and ^{125}I levels were determined in an LKB 1274 RIAGAMMA counter (LKB Instruments, Gaithersburg MD).

Retinol Binding Assay of Sertoli Cell-Conditioned Medium

Portions of conditioned media obtained from five Sertoli cell cultures were assayed for the presence of retinol-binding activity according to the procedure described by Carson et al. (1984). Briefly, to Sertoli cell-conditioned media, 2 μCi [15-^{3}H]retinol (51 Ci/mmol), 14 μg/ml of cholesterol (to compete for nonspecific lipid binding), and 50 μg/ml of alpha-tocopherol (as an antioxidant) were added to a final volume of 1.0 ml. The mixture was allowed to sit under N_2 in the dark at room temperature. After a 16-h incubation, unbound retinol was removed by adding 0.1 ml of a freshly prepared suspension of 0.5% (w/v) activated charcoal containing 0.1% Dextran T110 (Pharmacia, Piscataway, NJ). After another 10-min incubation at room temperature, the mixture was centrifuged for 10 min at 600 × *g* to sediment the charcoal-[^{3}H]retinol complex from protein-bound [^{3}H]retinol. The presence of a [^{3}H]-retinol-binding complex in the supernatant was assessed on a Sepharose CL-6B column (1.0 × 60 cm) that had been equilibrated with 0.3 M sodium chloride, 20 mM sodium phosphate, pH 7.0, containing 1 mM dithiothreitol, and 0.02% (w/v) sodium azide. The column was run with a flow rate of 0.25 ml/min at room temperature. Fractions of 0.45 ml were collected, and 0.10 ml aliquots of each fraction

were added to 10 ml of Hydrofluor scintillation counting solution (National Diagnostics, Somerville, NJ) for detection of [^{3}H] retinol in a Packard Tricarb Liquid Scintillation Counter (Packard Inst. Inc., Downers Grove, IL).

Other Assays

RBP was assayed in some Sertoli cell-conditioned media by a specific radioimmunoassay, which has been described previously (Muto et al., 1972; Smith et al., 1980). Total protein in the various samples was measured by the procedure of Lowry et al. (1951) using a mixture of bovine serum albumin and gamma globulins as standard.

RESULTS

Distribution and Levels of CRBP and CRABP in Various Rat Testis Cells

The distribution and levels of CRBP and CRAPB in cells isolated from rat testis seminiferous tubules were examined by specific and sensitive radioimmunoassays for each protein. Seminiferous tubules were isolated from 20- to 22-day-old male Wistar rats. These seminiferous tubules were separated into peritubular and Sertoli cells. The Sertoli cell isolates consisted of two distinct types of preparations, one that still contained germ cells and one devoid of germ cells (see Materials and Methods). Table 1 gives the distribution and levels of CRBP and CRABP measured in whole, freshly isolated seminiferous tubules, cultured (for 2 to 3 days) peritubular cells, and the two populations of cultured (for 3 days) Sertoli cells. Substantial CRBP levels were detected in whole, freshly isolated seminiferous tubules and in each of the three different cell populations. No significant differences in CRBP levels were observed between cultured Sertoli cells that contained germ cells and those free of germ cells. CRABP levels were high in whole seminiferous tubules and moderately high in Sertoli cell preparations that also contained germ cells. In contrast, the peritubular cell and germ cell-free Sertoli cell preparations contained low levels of CRABP.

Table 2 gives the CRBP and CRABP levels measured in preparations of spermatocytes and spermatids isolated from 34- to 36-day-old male rats. CRBP and CRABP were detected in both germ cell populations. The CRABP levels were substantially higher than the CRBP levels in both germ cell populations.

The effects of time in culture on the levels of CRBP and CRABP found in Sertoli cell cultures are given in Figure 1, both for the germ cell-containing (Fig. 1A) and the germ cell-free (Fig. 1B) Sertoli cell preparations. For this experiment, cells derived from the same cell preparation were cultured in different Petri dishes that were taken for assay at the time intervals indicated in this figure. Inspection of the data (Fig. 1) shows that CRBP and CRABP levels in cultures of Sertoli cells did not change substantially with time in culture. An analysis of variance (ANOVA) of these data indicates that CRBP and CRABP levels in the germ cell-containing Sertoli cells (Fig. 1A) did not change in a statistically significant manner with increasing time in culture and remained approximately constant for up to 7 days in culture. Similarly, the ANOVA indicates that the number of days in culture did not significantly affect the levels of CRBP and CRABP in Sertoli cells free of germ cells (Fig. 1B).

Absence of Retinol-Binding Protein Activity in Sertoli Cell-Conditioned Medium

To ascertain if conditioned medium from Sertoli cell preparations contained a retinol-binding protein similar to that reported by Carson et al. (1984) in conditioned medium from the Sertoli cell-derived TM-4 line, media from cultures of Sertoli cells were assayed according to the procedure described by Carson et al. (1984). The assay procedure consisted

TABLE 1. Distribution and levels of cellular retinol- and cellular retinoic acid-binding proteins in seminiferous tubules, peritubular cells, and Sertoli cells.

Tissue or Cell Type	(n)	CRBP[a]	CRABP[a]
		μg/mg protein	
Seminiferous tubules	2	1.19	1.81
Peritubular cells	8	1.48 ± 0.87	N.D.[b]
Sertoli + germ cells[c]	6	0.93 ± 0.24	0.81 ± 0.32
Sertoli − germ cells[c]	4	1.11 ± 0.20	0.16 ± 0.03

[a]Values are given as mean ± 1 SD, for the number (n) of different preparations listed. For the tubules (n=2), only the mean values are listed.

[b]N.D. means not detected. Only five of the eight peritubular cell preparations were assayed for CRABP. CRABP was not detected in any of these preparations. The detection limit for CRABP in these assays was 0.07 μg CRABP/mg protein.

[c]Assays were conducted by using Sertoli cells cultured for three days after isolation. Sertoli cell preparations, containing (+) germ cells and without (−) germ cells, were prepared as described in "Materials and Methods."

TABLE 2. Levels of cellular retinol- and cellular retinoic acid-binding protein in spermatocytes and spermatids.

Germ cell type	(n)	CRBP[a]		CRABP[a]	
		μg/mg protein	ng/10^6 cells	μg/mg protein	ng/10^6 cells
Pachytene spermatocytes	4	0.12 ± 0.10	31 ± 26	0.72 ± 0.26	181 ± 66
Spermatids	5	0.16 ± 0.05	12 ± 4	0.65 ± 0.21	49 ± 15

[a]Values are given as mean ± 1 SD for the number (n) of different preparations listed.

of incubation of the conditioned media with [^{3}H]-retinol and subsequent gel filtration on Sepharose CL-6B (see Materials and Methods). A typical elution profile of Sertoli cell-conditioned medium, after incubation with [^{3}H] retinol, is shown in Figure 2. Similar elution profiles were obtained with conditioned media from both the germ cell-containing and germ cell-free Sertoli preparations. Radioactivity (i.e. [^{3}H] retinol) eluted with the salt peak and not with proteins of small or large molecular weight, as shown in Figure 2. In no instance was a retinol-binding protein that eluted with the void volume of the Sepharose CL-6B column observed. A small number of conditioned media were also examined by radioimmunoassay for the presence of CRBP, CRABP, and RBP. Small amounts of both CRBP and CRABP were found in all media examined; however, the possibility that these findings resulted from a small amount of breakage could not be discounted. RBP was not detected in any medium examined.

DISCUSSION

Recent immunohistochemical studies (Kato et al., 1985a; Porter et al., 1985) have reported the striking localization of CRBP within the Sertoli cells of the testis. Earlier work by Huggenvik and Griswold (1981), employing a sucrose gradient assay, had shown that CRBP-binding activity was present in cultured Sertoli and peritubular cells. The present

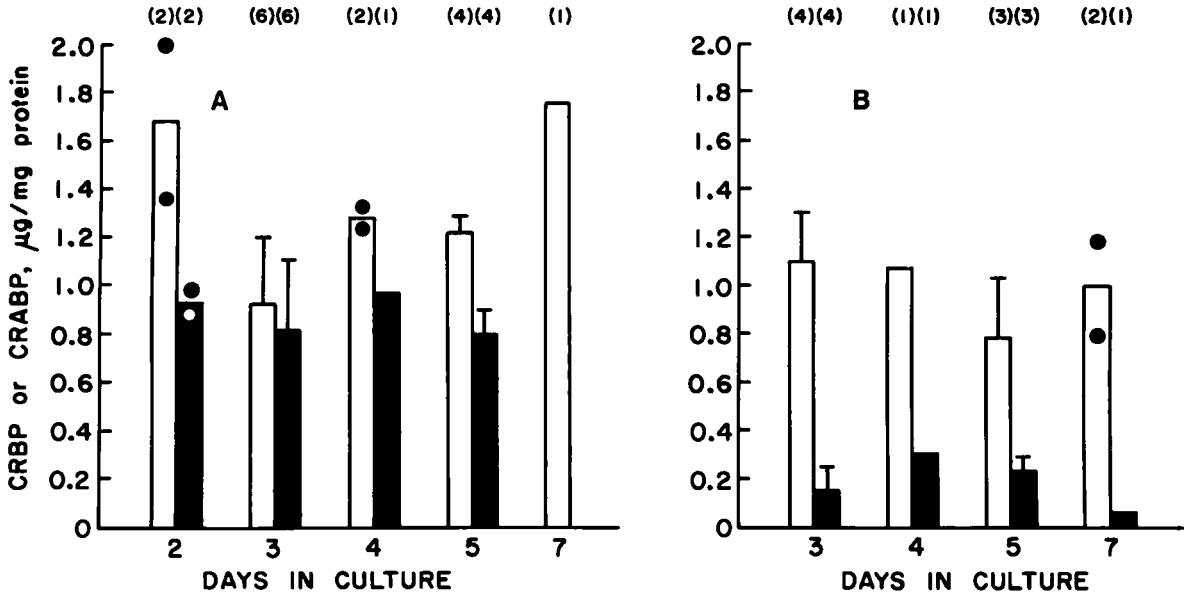

FIG. 1. Effects of the number of days in culture on CRBP and CRABP levels. *A*) Mean levels of CRBP and CRABP in a cultured cell preparation of Sertoli cells that also contain germ cells. *B*) Mean levels of CRBP and CRABP in cultured Sertoli cells that were shocked osmotically to destroy germ cells. The *numbers* listed above each *bar* give the number of cultures examined, and each *error bar* represents one standard deviation. In instances where only two cultures were examined, the individual levels of CRBP and CRABP measured in each culture are given by the *circles*. *Open bars* give CRBP levels; *solid bars* give CRABP levels.

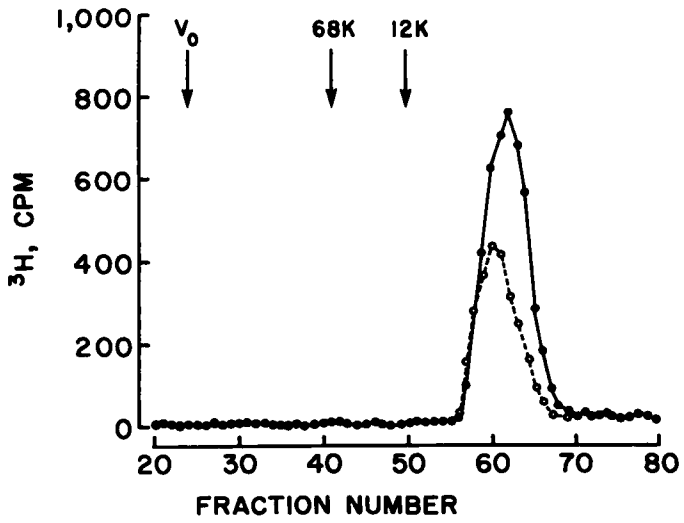

FIG. 2. Gel filtration of conditioned media on a column of Sepharose CL-6B. The details of the conditions are described in *"Materials and Methods."* ——o—— Media from Sertoli cells containing germ cells; —•— media from germ cell-free Sertoli cells. Approximately 2–3 mg protein was applied to each column. V_0 indicates the void volume. The *68K* marker was bovine serum albumin, and the *12K* marker was cytochrome c.

study confirms and extends these previous studies, and provides precise quantitative data on the levels of CRBP in Sertoli and peritubular cells, as determined by radioimmunoassay. High levels of CRBP were found in both cell types, with the mean level of CRBP in peritubular cells (1.48 μg/mg protein) approximately 50% higher than the level found in Sertoli cells (when expressed in these units).

The information available concerning the distribution of CRABP in the rat testis is much more limited than that for CRBP. Porter et al. (1985) recently reported the immunohistochemical localization of CRABP in the testis. These workers found no positive staining for CRABP in the Sertoli cells, whereas late spermatocytes and spermatids showed positive staining for CRABP. The present study provides quantitative information, determined by radioimmunoassay, on the levels of CRABP in different types of testis cells. No CRABP was detected in cultured peritubular cells. Low levels of CRABP (mean 0.16 μg/mg protein) were found in Sertoli cells that were free of germ cells. Much higher levels of CRABP (approximately 0.7 μg/mg protein) were found in isolated germ cells (spermatocytes and spermatids). Thus, whereas Sertoli cells are highly enriched in CRBP, germ cells are highly enriched in CRABP. It is likely that much of the CRABP observed in the Sertoli cell preparations that contained germ cells arose from the germ cells present in those preparations.

It should be emphasized that the Sertoli cell preparations were isolated from 20- to 22-day-old rats, whereas the spermatids and pachytene spermatocytes were isolated from 34- to 36-day-old rats. The germ cells associated with these isolated Sertoli cells are spermatogonia and primary spermatocytes. Thus one should be cautious about directly comparing CRBP or CRABP levels as measured in isolated spermatocytes and spermatids with those measured in the isolated Sertoli cells. It is, however, clear that since Sertoli cells containing germ cells are greatly enriched in CRABP level compared to germ cell free-Sertoli cells, the germ cells associated with the Sertoli cells are probably enriched in CRABP. Porter et al. (1985) have reported that CRABP is primarily associated with late stage germ cells, from pachytene spermatocytes to spermatids. We cannot readily reconcile our data, which suggest that spermatogonia and primary spermatocytes are also relatively enriched in CRABP, with those of Porter et al.; however, this discrepancy may reflect a difference in methodology (immunohistochemistry vs. radioimmunoassay).

Germ cells were observed by Kato et al. (1985a) to manifest weak immunohistochemical staining for CRBP, whereas Porter et al. (1985) observed no immune staining for CRBP in the germ cells. Previously, Huggenvik and Griswold (1981), using a relatively insensitive sucrose gradient assay, could not detect CRBP-binding activity in isolated germinal cell preparations consisting primarily of spermatids and spermatocytes. As shown in Table 2, both pachytene spermatocytes and spermatids contained CRBP and CRABP. However, the levels of CRABP were approximately four to five times greater than the levels of CRBP in both the spermatocytes and spermatids. The roles of these two binding proteins in the germ cells ramain to be elucidated. It has been reported that both retinol and retinoic acid influence spermatogenic differentiation in the cryptorchid mouse testis (Haneji et al., 1984). In view of the high level of CRABP found in the germ cells, it is tempting to speculate that retinoic acid might play some particular role in spermatogenic differentiation.

We found that both CRBP and CRABP levels remained relatively constant for at least seven days in cultured Sertoli cells. This finding was true for both the Sertoli cell preparation that contained germ cells and the preparation that was free of germ cells. This

stability of CRBP and CRABP levels suggests that the Sertoli cell levels reported in Table 1, determined on cells that had been cultured for three days, were also representative of the levels of the binding proteins that had been present in the Sertoli cells in vivo. This stability in the binding protein levels in Sertoli cells suggests the possibility of developing a model system for studying the effects of hormones and other factors on CRBP and CRABP levels and metabolism in these cells. Along these lines, Huggenvik and Griswold (1981) reported that FSH and FSH and testosterone together elevated CRBP-binding activity levels in cultured Sertoli cells. It is possible that cultured primary Sertoli cells will be useful for exploring the poorly understood factors that regulate CRBP and CRABP levels.

As part of this study, we attempted to determine if Sertoli cells in primary culture secrete a relatively high molecular weight binding protein for retinol, distinct from RBP and CRBP, into the medium. The secretion of such a binding protein has been reported by Carson et al. (1984) for the murine Sertoli-derived TM-4 cell line. Carson et al. (1984) suggested that this protein might be involved in the transport of retinol to the lumen of the seminiferous tubules or to the developing germ cells themselves. Conditioned media from five primary Sertoli cell cultures (both containing germ cells and free of germ cells) were examined on Sepharose CL-6B columns for the presence of such binding-protein activity. We could not detect this binding protein in any of the media examined. The reason for this difference between our observations and the report of Carson et al. (1984) is not clear. It should be noted that the TM-4 cells were maintained in medium supplemented with FSH, insulin, and testosterone, whereas the medium used here for Sertoli cell culture was free of these additions. A limited number of Sertoli cell-conditioned medium samples were also examined by radioimmunoassay for the presence of RBP, CRBP, and CRABP. RBP was not detected in any medium examined. CRBP and CRABP were present at low levels in all media examined. The levels of both CRBP and CRABP were quite variable, however, among the different cultures examined, and the possibility that the CRBP and CRABP in the media resulted from cell breakage could not be ruled out. Further study along these lines will be needed to determine if the small amounts of CRBP and/or CRABP found in the medium do arise from cell breakage, or whether these proteins might be released in small quantities from the intact cells under the conditions employed.

The transport of retinoids between the different cell types that compose certain organs is becoming the focus of much research. In the eye, the interphotoreceptor matrix retinol-binding protein (IRBP) is known to transport retinoids between the retinal pigment epithelium (where IRBP is synthesized) and the rod cells (where IRBP is internalized) (Bridges, 1984; Hollyfield et al., 1985). In the liver, retinol (or retinyl ester) must be transferred between the parenchymal cells (where chylomicron retinol is first internalized and where plasma RBP is synthesized) and the stellate cells (where retinol is stored) (Blomhoff et al., 1982, 1985; Blaner et al., 1985). Unlike in the eye, the mechanism of transfer of retinoids between the two liver cells types has not been elucidated.

In the testis, as in the eye and liver, retinoids must be transported between the different cellular components. Previous studies have shown that most of the CRBP and CRABP in the rat testis is present as its respective holo-protein, i.e. the binding protein containing a molecule of retinoid ligand (Ross et al., 1978, 1980). Accordingly, the level of a given binding protein in a particular cell population can be taken as an index of the level of the respective retinoid ligand in those cells. If this is true, then the data reported here indicate that both Sertoli and germ cells contain significant amounts of retinoids, with retinol the predominant retinoid in Sertoli cells and retinoic acid in the germ cells. Previous studies on the distribution on vitamin A in the testis have suggested that the Sertoli cell may be a significant site of localization of retinol (Ahluwalia et al., 1975; Rajguru et al., 1982). It is known that, because of the blood-testis barrier, all nutritional or regulatory substances have to pass through the Sertoli cell cytoplasm to reach germ cells within the adluminal compartment (Steinberger and Steinberger, 1977; Ritzen et al., 1981). Thus, retinoids in the germ cells had to have been transported into these cells from Sertoli cells. No information is available about the mechanism involved in this cell-cell transfer process within the seminiferous tubule. The presence of CRABP in Sertoli cells, but not in peritubular cells, suggests that Sertoli cells might be a site of formation of retinoic acid from retinol in the testis. Alternatively, retinoic acid might be produced mainly in germ cells, or might be selectively taken up by tubular cells from the blood (De

Leenheer et al., 1982). Similarly, the nature of the retinoid transferred, whether retinol or retinoic acid or both, remains to be determined. Further studies will be needed to explore these questions concerning retinoid metabolism in the testis.

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