

Sertoli Cell Secretion of a Factor that Inhibits the Incorporation of ^3H -Thymidine into Cells *In Vitro*

DOLORES J. LAMB,*†‡ SANG LEE,* AND SANKARARAMAN SHUBHADA*‡

From *the Scott Department of Urology and the †Department of Cell Biology, Baylor College of Medicine, Houston, Texas; and ‡The Methodist Hospital Center for Reproductive Medicine and Surgery, Houston, Texas.

ABSTRACT: Rat Sertoli cells secrete a low molecular weight factor *in vitro* that inhibits the incorporation of ^3H -thymidine into cells. Although the addition of Sertoli cell-conditioned medium (rSCCM) resulted in nearly a threefold stimulation of cell growth, the incorporation of ^3H -thymidine was decreased in a dose-dependent manner and did not reflect the increase in cell number. Peritubular cell- and germ cell-conditioned medium did not contain this inhibitory activity. Nor did the conditioned medium from fibroblasts and a variety of cell

lines tested. A low molecular weight filtrate of rSCCM (<1,000 Da) contained virtually all of the ^3H -thymidine inhibiting activity, as well as about 50% of the mitogenic activity in the rSCCM. The inhibitory activity was eliminated upon removal of the rSCCM and was not due to either growth inhibition or a toxic effect on cell proliferation.

Key words: Sertoli cell, cell proliferation, DNA synthesis.

J Androl 1994;15:110-116

The Sertoli cell, which is thought to provide nutrition and support to the developing germ cells, mediates the effects of follicle-stimulating hormone (FSH) and testosterone on spermatogenesis. Studies have focused on the role of polypeptide growth factors in the regulation of spermatogenesis (reviewed in Feig et al, 1980; Bellve and Feig, 1984; Hall et al, 1983; Holmes et al, 1986; Tres et al, 1986; Khan et al, 1987; Smith et al, 1987; Buch et al, 1988; Syed et al, 1988; Skinner and Moses, 1989; Skinner et al, 1989; Smith et al, 1989; Buch et al, 1991; Lamb et al, 1991); however, it is possible that paracrine regulation of cell function in the testis could occur via a number of additional pathways involving general metabolic processes and nucleoside utilization.

During the course of our studies on Sertoli cell-secreted growth factor (SCSGF) (Lamb et al, 1987, 1991; Lamb, 1993); we noted that Sertoli cell-conditioned medium (rSCCM) influenced the incorporation of ^3H -thymidine into cultured cells. In this report we demonstrate that rSCCM exhibits a dramatic inhibitory effect on the incorporation of this radiolabeled precursor into cells, yet there is an incongruous increase in cell proliferation.

Supported in part by National Institutes of Health grant DK 39719 (DJL).

Correspondence to: Dr. Dolores J. Lamb, Department of Urology, Room 440E, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

Received for publication August 20, 1992; accepted for publication September 2, 1993.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DME) and Hanks' balanced salt solution (HBSS) were purchased from GIBCO. Hyaluronidase and DNase were obtained from Sigma. Collagenase was from Cooper Biomedical and FSH was a gift from the National Hormone and Pituitary Program (NIADDK, NIH-FSH-0-14). Testosterone was purchased from Steraloids Inc. (Wilton, New Hampshire). The A431 cells and Swiss 3T3 cells were obtained from the American Type Culture Collection (ATCC). The TM₄ cells were provided by Dr. Patricia Morris (Population Council, Rockefeller University, New York). ^3H -thymidine (6.7 Ci/mmol) was purchased from Dupont/New England Nuclear.

Animal Care

All animals were housed under standard conditions of 12 hours light and dark and provided food and water *ad libitum*. Rats were sacrificed by CO₂ inhalation. This protocol was approved by the Animal Research Committee at Baylor College of Medicine in compliance with all applicable guidelines.

Sertoli Cell Isolation and Culture

Sertoli cells were isolated enzymatically and cultured using the protocol of Steinberger et al (1975), with the modifications of Tung (1984). After 2 days of culture in DME, the medium was exchanged for DME supplemented with insulin (5 $\mu\text{g}/\text{ml}$) and FSH (200 ng/ml). After three additional days of culture, the rSCCM was obtained and used in the cell growth assay and the ^3H -thymidine incorporation assay.

The first group of experiments used rSCCM from Sertoli cells plated at a concentration of five or six testes/dish (240-300 μg DNA/dish, about 40-50 $\times 10^6$ cells/dish) with 5 ml DME. Subsequent studies used Sertoli cells plated at 22-25 $\times 10^6$ cells/

Table 1. Effect of rSCCM and mEGF on ³H-thymidine incorporation into confluent Swiss 3T3 cells*

Growth factor	Concentration	³ H-thymidine (DPM × 10 ³)
0	0	33.4 ± 2.5
rSCCM	20%	4.9 ± 0.4†
	50%	3.5 ± 0.1†
	100%	3.1 ± 0.9†
mEGF	2.5 ng/ml	241.0 ± 10.0†
	5.0 ng/ml	273.0 ± 18.5†
rSCCM plus mEGF	50%, 5 ng/ml	10.6 ± 4.0†

* Confluent Swiss 3T3 cells were incubated for 18 hours in 2% calf serum DME and increasing concentrations of rSCCM (five-sixth testes/dish) and in EGF alone or in combination. The cells were pulsed for 1 hour with 1 μ Ci/ml ³H-thymidine and the acid-precipitable incorporation of ³H-thymidine counted in the liquid scintillation counter.

† $P < 0.001$.

dish in 7 ml DME (lower cell density), as specified in the figure legends.

Sertoli cell cultures contained >90–95% Sertoli cells. Estimates of purity were based on morphological appearance and cytochemical assays. Staining with nitrobluetetrazolium showed no evidence of 3- β -ol-hydroxysteroid dehydrogenase activity, which would indicate the presence of Leydig cells (Tcholakian and Steinberger, 1978). Immunocytochemical staining for desmin showed no evidence of peritubular cells (Anthony and Skinner, 1989).

Peritubular Cell Isolation and Culture

Peritubular cells were isolated from the collagenase/hyaluronidase digestion supernatant (the Sertoli cell aggregates were separated from the peritubular cells by gravity sedimentation). The cells were centrifuged, washed, and plated in 10% calf serum. The cells were allowed to grow to confluence in 100-mm plates prior to subculturing at 25% confluence. The peritubular cells were again allowed to become confluent (about 75×10^6 cells/dish; 3–4 days of culture), and at this time the medium was replaced with serum-free DME and conditioned medium (PTCM) obtained after an additional 3 days of culture as described for the Sertoli cells.

Unlike the Sertoli cells, the peritubular cells proliferated and had a characteristic fibroblast-like appearance. About 30% of the peritubular cells stained positively for desmin (Anthony and Skinner, 1989), a value that is slightly lower than the 40% reported by Anthony and Skinner using similar culture conditions (1989).

Germ Cell Isolation

For germ cell isolation, decapsulated testes from six 35-day-old rats were incubated in 0.1% collagenase at 37°C for 40 minutes. The tubules were allowed to sediment, washed three times with HBSS and minced in 0.2% trypsin and 2 μ g/ml DNase. The supernatant containing the dispersed germ cells was passed through two layers of lens paper, washed twice with 0.2% bovine serum albumin (BSA) in HBSS and cultured in DME (5×10^6 cells/cm²). Based upon phase contrast microscopy and aceto-orcein staining (Galliger and Kozloff, 1971; Scully et al, 1987),

the germ cells were primarily a heterogeneous mixture of pachytene spermatocytes and round spermatids.

Cell Lines Used

We chose to use A431 cells (derived from a human epidermoid carcinoma; Giard et al, 1973) because they have a rapid doubling time, epidermal growth factor (EGF) inhibits their growth, and SCSGF is the only known mitogen for this cell type (Lamb et al, 1991). We routinely use this cell type for studies of testicular mitogens. Conversely, Swiss 3T3 cells, frequently used for ³H-thymidine incorporation studies, are stimulated by EGF to incorporate ³H-thymidine. The murine TM₄ cell line was chosen for some of these studies because it is Sertoli-cell like (Mather, 1980).

Cell Growth Assay

A431 cells were chosen for the cell growth assay. These cells have a rapid doubling time that makes them useful for studies requiring proliferation. This assay was described by Holmes et al (1986) and will briefly be described. A431 cells were plated in 1% fetal bovine serum (FBS) in DME overnight. The medium was removed, and various concentrations of samples were added to each dish as specified. The cells were cultured for an additional 3 days and removed with 0.25% trypsin, 0.1 mM EDTA for counting in a Coulter Counter. All conditions were tested in triplicate.

³H-Thymidine Incorporation Assay

The cells (Swiss 3T3, A431, or TM₄) were incubated with ³H-thymidine (1 or 5 μ Ci/ml) as described in the figure legends. The cells were then washed three times with ice-cold phosphate-buffered saline (PBS) and removed from the dishes with 0.1% trypsin-EDTA. The cells were harvested with an automatic cell harvester (Brandis) onto glass fiber filters. The acid-insoluble protein and DNA were precipitated with ice-cold 10% trichloroacetic acid (TCA) and washed with 3 volumes to remove the acid-soluble fraction. The glass fiber filters were counted for 10 minutes each in 10 ml of Scintiverse in the scintillation counter (Beckman).

Swiss 3T3 cells were also used for a number of the ³H-thymidine incorporation studies. These cells were chosen because they are stimulated to incorporate ³H-thymidine by mouse epidermal growth factor (mEGF), which served as a positive control. The 3T3 cells were cultured in DME supplemented with 2% calf serum. During logarithmic growth, the cells were incubated overnight with rSCCM or partially purified inhibitory factor followed by labeling for 1 hour with ³H-thymidine (1 μ Ci/ml). Cells were cultured in multi-well plates.

For the 96-well plates 6×10^3 cells were plated per well and allowed to attach for 24 hours. The medium was replaced with the reagents to be tested in 200 μ l and cultured overnight. After labeling with ³H-thymidine (1 μ Ci/ml), the monolayers were washed with PBS and fixed in methanol:acetic acid (3:1; 200 μ l/well) for 5 minutes at room temperature. Following a subsequent wash with methanol (5 minutes, room temperature), the plates were placed on ice and washed with 5% TCA (2°C, 5 minutes), followed by three washes with methanol. After air drying the plates, the monolayers were hydrolyzed in 200 μ l 1 N NaOH (5

Table 2a. Effect of rSCCM on incorporation of ^3H -thymidine into confluent TM_4 cells*

	rSCCM (%)	^3H -thymidine (DPM $\times 10^3$)
Control	0	274 \pm 22.0
rSCCM	20	216 \pm 20.0
	50	73 \pm 8.0†
	100	3 \pm 1.0†

* Confluent TM_4 cells were cultured for 24 hours with rSCCM and DME, 25% F12, 2.5% horse serum, and 1.25% FBS. Cells were pulsed with 1 μCi /well ^3H -thymidine for 1 hour.

† $P < 0.05$.

Table 2b. Effect of rSCCM on TM_4 cell proliferation*

	rSCCM (%)	TM_4 cells ($\times 10^3$)
Control	0	225 \pm 24
rSCCM	50	400 \pm 20*†

* TM_4 cells were plated at 14×10^3 cells/dish, then cultured with 50% rSCCM for 3 days. Cells were collected by trypsinization and counted. rSCCM was obtained from Sertoli cells plated at five-sixth testes/dish.

† $P < 0.05$.

minutes, room temperature), and 180- μl aliquots were counted in the scintillation counter.

Statistical Analysis of Data

Each experiment was reproduced two to three times. Each sample was tested in triplicate and results expressed as mean \pm SD. Statistical significance was determined by Duncan's multiple range test and one-way analysis of variance. Although some variation in the amount of ^3H -thymidine inhibiting activity was observed between different primary Sertoli cell cultures as described in the text, appropriate controls were included in each experiment.

Results

rSCCM Inhibition of ^3H -Thymidine Incorporation into Swiss 3T3 Cells

When confluent Swiss 3T3 cells were incubated overnight with rSCCM and pulsed for 1 hour with ^3H -thymidine, an approximately 90% decrease in ^3H -thymidine incorporation was observed (Table 1). Mouse EGF (2.5 or 5 ng/ml) was stimulatory, but the addition of 50% rSCCM totally inhibited the EGF-induced stimulation of incorporation. Over a dose range of 0.1 to 10 ng/ml EGF, the addition of 50% rSCCM dramatically inhibited ^3H -thymidine incorporation. The inhibition of ^3H -thymidine incorporation by rSCCM was observed not only in confluent but also in rapidly growing Swiss 3T3 cells and TM_4 cells (Table 2a). In contrast, this medium was mitogenic in a cell growth assay (Table 2b). Interestingly, TM_4 cell-

Table 3. Effect of TM_4 cell conditioned medium on the incorporation of ^3H -thymidine into confluent Swiss 3T3 cells*

Treatment	Concentration (%)	^3H -thymidine incorporation
Control	0	38.9 \pm 1.5
Calf serum	10	95.6 \pm 12.8†
rSCCM	10	5.9 \pm 1.6‡
	20	4.2 \pm 0.4‡
	50	4.9 \pm 0.1‡
TM_4 CM	10	40.4 \pm 3.6
	20	36.5 \pm 3.5
	50	28.0 \pm 7.8
TM_4 CM plus rSCCM	25, 25	3.3 \pm 0.3‡

* Swiss 3T3 cells were grown to confluence and incubated overnight with rSCCM (obtained from Sertoli cells plated at five-sixth testes/dish), EGF, or DME (control) in 2% calf serum. After a 1-hour labeling period with 1.0 μCi ^3H -thymidine, the wells were trypsinized and the cells were precipitated with 5% TCA and collected on glass filters.

† $P < 0.05$.

‡ $P < 0.01$.

conditioned medium had no effect on ^3H -thymidine incorporation into confluent Swiss 3T3 cells (Table 3). In addition, conditioned medium from A431 cells, male genital skin fibroblasts, and other cell lines (DDT₁MF-2, R23327H-G8A1) tested did not contain the ^3H -thymidine inhibiting activity (data not shown). The ^3H -thymidine inhibiting activity was present in the conditioned medium from Sertoli cells cultured from all ages tested (days 18–60).

The rSCCM inhibited ^3H -thymidine incorporation into Swiss 3T3 cells in a dose-dependent manner (Fig. 1). Figure 1 shows data pooled from separate experiments using rSCCM from 13 different primary Sertoli cell cultures and contrasts the relative amount of inhibitory activity secreted when the Sertoli cells are plated at two different densities. Thus, the relative secretion of the inhibitory factor was remarkably consistent between experiments, but it varied in response to the initial Sertoli cell plating conditions. These conditions are specified in each figure legend.

Testicular Cell Specificity of Secretion

Peritubular cells were isolated and cultured from 18- to 21-day-old rats, and after one passage to remove any contaminating Sertoli cells, the conditioned medium was obtained exactly as described for the Sertoli cells. The effect of this medium on the incorporation of ^3H -thymidine into A431 cells was tested. Figure 2a shows that peritubular cell-conditioned medium did not contain any of the ^3H -thymidine inhibiting activity. Thus, any fibroblasts contaminating the Sertoli cell cultures were not the source of this activity. Similarly conditioned medium obtained from isolated germ cells did not contain the inhibitory activity (Fig. 2b).

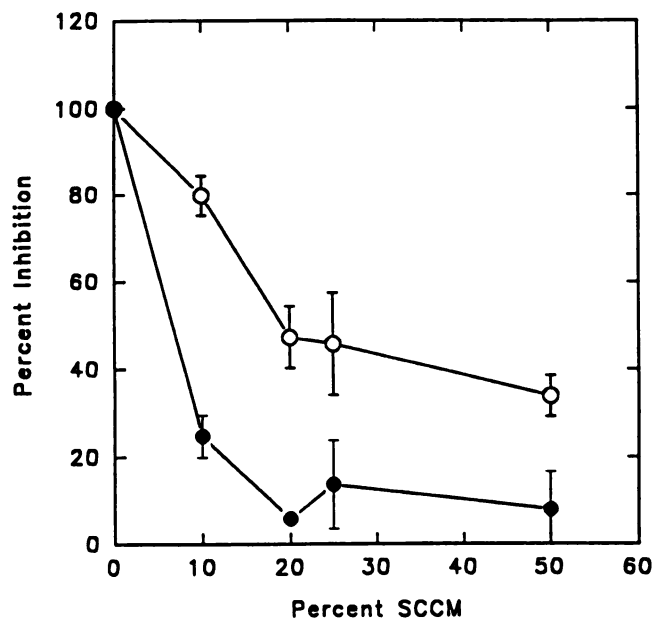


FIG. 1. Inhibition of ³H-thymidine incorporation into Swiss 3T3 cells during the early log phase of growth. Swiss 3T3 cells (5×10^3 cells) were plated in 5% FBS DME for 24 hours. The cells were incubated for 24 hours in 2% calf serum DME with increasing concentrations of rat Sertoli cell-conditioned medium (rSCCM). The results from rSCCM obtained from Sertoli cells plated at five-sixth testes/dish with 5 ml medium (●) and from Sertoli cells plated at $22-25 \times 10^6$ cells/dish, 7 ml medium (○), are contrasted. After a 1-hour incubation with ³H-thymidine, followed by precipitation with 5% TCA, the samples were dissolved in 0.1 N NaOH and counted in the scintillation counter. Results of seven experiments using conditioned medium from Sertoli cells plated at five or six testes/dish and six experiments with Sertoli cells plated at the lower density are expressed as mean \pm SD.

Ultrafiltration of ³H-Thymidine Inhibiting and Mitogenic Activities in rSCCM

rSCCM was subjected to ultrafiltration using an Amicon YM-2 membrane, and the filtrate (containing only those factors with a molecular weight of $<1,000$) and the original untreated rSCCM were tested in both the cell growth assay for mitogenic activity and the ³H-thymidine incorporation assay (Fig. 3). In the lower panel of Figure 3, the results of the ³H-thymidine incorporation assay show that the filtrate contained a virtually identical concentration of inhibiting activity, compared with the starting rSCCM, over a concentration range of 10–50%. In contrast, about 45% of the mitogenic activity was lost from the filtrate and thus had a molecular weight of $>1,000$. However, the results also show that there was a significant amount of low molecular weight mitogenic activity in the rSCCM. Nevertheless, this low molecular weight medium fraction inhibited the incorporation of ³H-thymidine into cells.

Stability of Inhibitor Activity

rSCCM filtrate was treated with heat (100°C, 10 minutes), acid (pH 3.0, 1 hour, room temperature), and trypsin

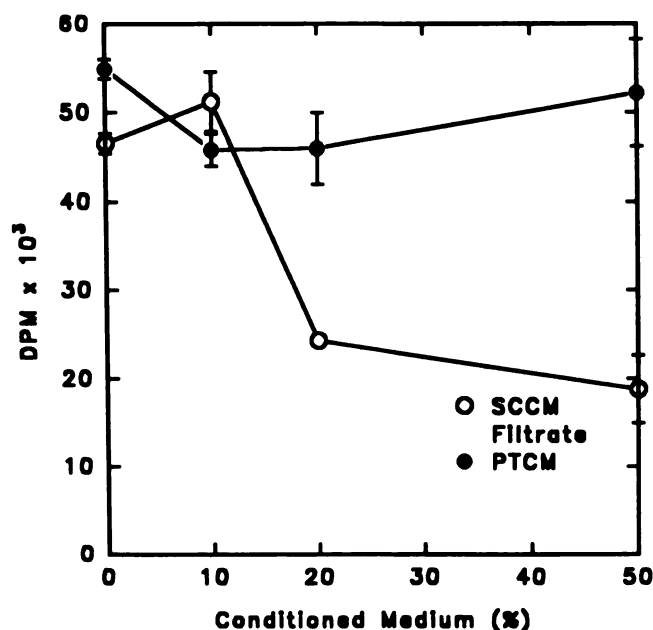
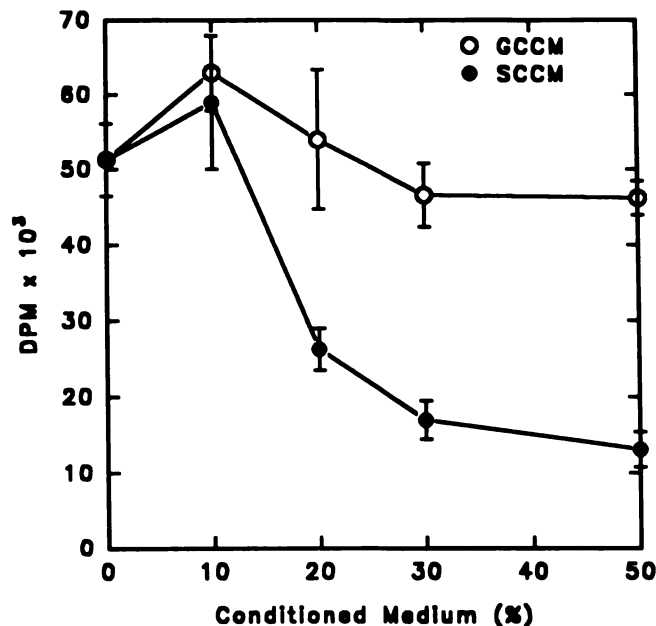


FIG. 2. Cellular specificity of the secretion of the ³H-thymidine incorporation inhibiting activity. Peritubular cells were cultured from 18- to 21-day-old rats in 10% FBS DME. After one passage to remove contaminating Sertoli cells, conditioned medium was obtained as described for Sertoli cells. In the top panel, germ cell-conditioned medium (GCCM) was tested over a dose range of 0–50% in the ³H-thymidine incorporation assay. Results are expressed as percent of DME control. The lower panel shows the absence of effect of peritubular cell-conditioned medium (PTCM) over the same concentration range.

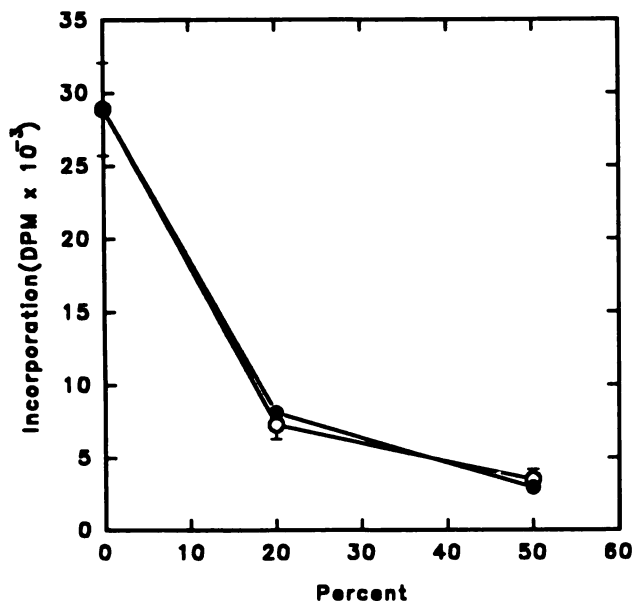
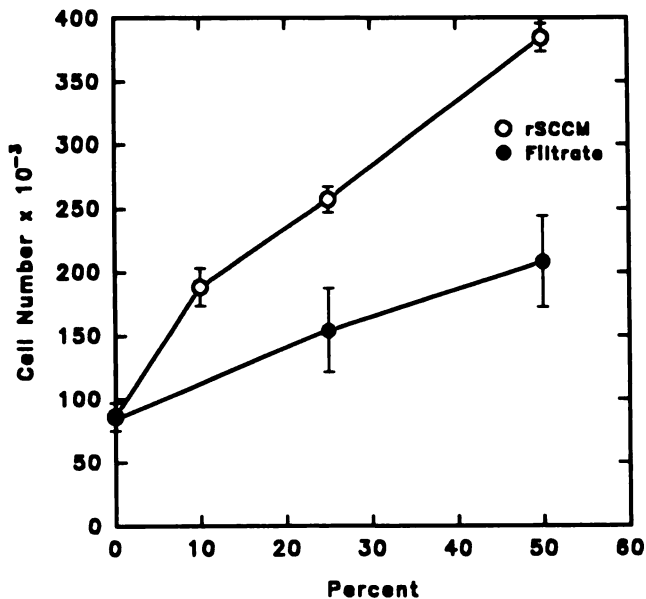


FIG. 3. Ultrafiltration of the ^3H -thymidine inhibiting and mitogenic activities in rSCCM. rSCCM was filtered through an Amicon YM-2 membrane. The starting rSCCM (○) and the low molecular weight filtrate (●) containing factors with molecular weights <1,000 were tested in the A431 cell growth assay (top panel) and the ^3H -thymidine incorporation assay (bottom panel).

(0.25%, 3 hours, 37°C) prior to addition to the Swiss 3T3 fibroblasts. The incorporation of ^3H -thymidine was equally inhibited after all treatments, demonstrating that the activity is heat-, acid-, and trypsin-stable (Fig. 4). Control DME exposed to the same treatments was not inhibitory (not shown).

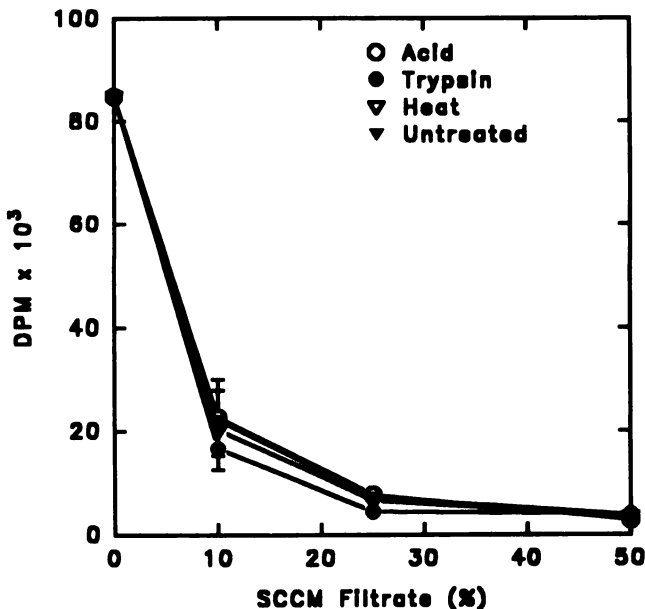


FIG. 4. Stability of the inhibitory activity. rSCCM filtrate (five-sixth testes/dish) and DME control filtrate (not shown) were treated with acid (acetic acid, pH 3.0, 1 hour, room temperature), heat (100°C, 10 minutes), and trypsin (0.25%, 3 hours, 37°C) followed by addition of trypsin inhibitor to neutralize the enzymatic activity. The filtrates were lyophilized and reconstituted prior to use in a ^3H -thymidine incorporation assay using Swiss 3T3 cells. The samples were treated over a concentration range of 0–50%. Treatments of the DME controls had no effect on ^3H -thymidine incorporation and are not shown. Samples were tested in triplicate and results are expressed as the mean \pm SD. Error bars where not apparent are smaller than the size of the symbol.

Time Course of ^3H -Thymidine Labeling in the Presence of rSCCM

Because the cell growth and ^3H -thymidine incorporation assays were performed under different experimental conditions, rSCCM was tested for mitogenic and ^3H -thymidine inhibiting activities in the same experiment using A431 cells. In addition, the labeling time with ^3H -thymidine was varied over an 18-hour period to rule out any effects that rSCCM might have on equilibration of ^3H -thymidine with the intracellular pools of thymidine in the cells. Table 4 shows the time course of labeling the A431 cells with ^3H -thymidine. Incubation of the cells with rSCCM for 3 days inhibited the incorporation of ^3H -thymidine into rapidly growing A431 cells after a 1-, 2-, 6-, or 18-hour pulse, yet over this 3-day incubation period with rSCCM, these cells were stimulated to grow nearly threefold over control values. One group (2 \times) treated with rSCCM received fresh rSCCM 18 hours prior to the end of the experiment. No further inhibition of ^3H -thymidine incorporation was observed as compared with the group treated with one application 3 days prior to the assay.

Figure 5 shows that the inhibitory effect is fully reversible upon removal of the conditioned medium. Swiss 3T3 cells were incubated with increasing concentrations

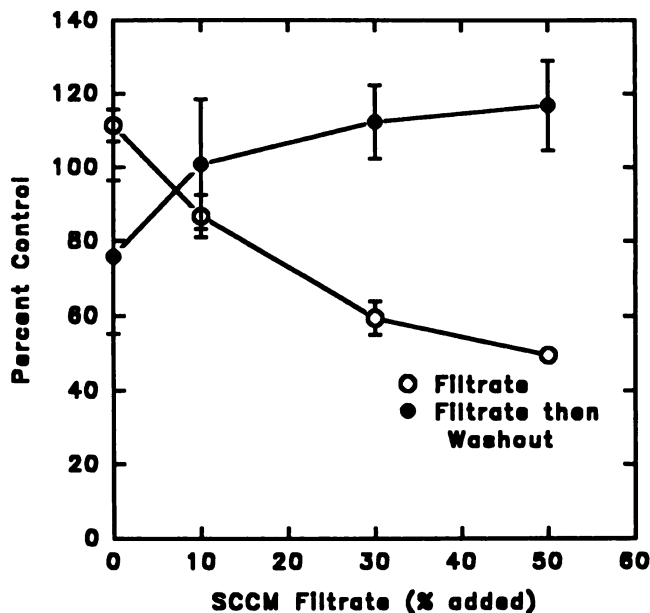


FIG. 5. Reversibility of inhibitory action of rSCCM filtrate on ³H-thymidine incorporation. Swiss 3T3 cells were plated in 96-well plates and incubated overnight with increasing concentrations of rSCCM filtrate (<500 Da molecular weight; 22–25 × 10⁶ Sertoli cells/dish). Half of each test group was washed with HBSS prior to the addition of ³H-thymidine in fresh DME with 2% calf serum (filtrate then washout, ●). ³H-thymidine was added directly to the other set of dishes without a medium change (○). Each sample was tested in triplicate and results expressed as mean ± SD. The error bars for the filtrate 50% group were smaller than the symbol.

of rSCCM filtrate (<500-Da molecular weight) for 24 hours. One group of dishes was washed extensively with HBSS (washout) prior to the addition of ³H-thymidine in fresh DME; the other group had ³H-thymidine added directly to the cultures without a medium change. The inhibitory factor was removed by washing the cells.

Discussion

During the course of our studies on mitogens secreted by the Sertoli cell, conflicting results were obtained from our growth assays and the ³H-thymidine incorporation assays. That is, although incubation of cells with rSCCM resulted in an increase in cell proliferation, the incorporation of ³H-thymidine into cells was paradoxically decreased. The results show that the inhibitory effect of rSCCM on ³H-thymidine incorporation was not due to an effect on the incubation time required for equilibration of thymidine into the intracellular pools. Based on filtration experiments, it is low molecular weight (<1,000 Da) and heat-, acid-, and trypsin-stable (Table 4).

We and others have demonstrated that Sertoli cell-conditioned medium is mitogenic for a variety of cultured cell lines (reviewed in Buch et al, 1991), including the cell lines used in the present study. Accordingly, the inhibitory

Table 4. Time course of ³H-thymidine labeling of A431 cells: effect of rSCCM*

	Control	rSCCM (33%)	rSCCM2x (33%)
No. of cells (× 10 ³)	142 ± 18	428 ± 22	450 ± 22
³ H-thymidine labeling time (hours)	³ H-thymidine incorporation (DPM/cell)		
1	0.25	0.34	0.15
2	0.78	0.40	0.34
6	1.70	0.75	0.73
18	3.03	1.14	0.86

* Sertoli cells were isolated and cultured from 36-day-old rats. After 2 days of culture (five-sixth testes/dish; 5 ml medium per dish), the medium was replaced with fresh DME containing FSH (200 ng/ml) and insulin (5 μg/ml). A431 cells were plated in 5% FBS for 24 hours prior to serum-free culture in DME (control) or rSCCM (33%). One complete set of dishes was incubated with rSCCM for 3 days, and another set was incubated with rSCCM for 3 days, with a second application at 18 hours prior to the end of the incubation. The cells were incubated with ³H-thymidine 1 μCi/well for various time periods. Replicate wells were used to determine cell number.

activity measured in the ³H-thymidine incorporation was not due to the presence of a growth inhibitory peptide. Furthermore, the inhibitory effect was not due to either a toxic action of the rSCCM on the proliferating cells or to an accompanying inhibition of cell proliferation. In addition, the DNA content per cell was not altered in the presence of this Sertoli cell activity (data not shown), and there is no evidence to suggest that normal *de novo* synthesis of thymidine does not occur in the presence of rSCCM.

The cell normally synthesizes thymidine *de novo* through a pathway converting dUMP to dTMP using thymidylate synthetase. When thymidine is added to culture medium, it enters the cell through a salvage pathway into the acid-soluble nucleoside pool of the cell and is successively phosphorylated and incorporated in DNA. Incorporation reflects the uptake of precursor, the specific activity of the nucleoside pools within the cells, and the relative amount of ongoing DNA synthesis. There are several obvious steps in nucleoside uptake/biosynthesis that may be affected by this Sertoli cell-secreted activity. The uptake of thymidine into the cell and/or the subsequent phosphorylation may be altered (thymidine → TMP → TDP → TTP). Alternatively, the salvage pathway may not be utilized in the presence of the Sertoli cell-inhibiting factor. Cultured cells do not normally secrete thymidine into culture medium, and the present study has demonstrated that other cultured testicular cell types, including peritubular cells and germ cells, as well as A431 cells from a human epidermoid carcinoma of the vulva, genital skin fibroblasts, and the male reproductive tumors tested (DDT₁MF-2 and R23327H-G8A1) did not contain this inhibitory activity.

Cell incubation with radiolabeled thymidine (or other DNA or RNA precursors) has frequently been used to measure the effects of steroid and peptide hormones, growth factors, growth inhibitory factors, and other agents on cell proliferation and RNA synthesis. The results suggest that great caution must be used with the interpretation of this type of assay because the ^3H -thymidine incorporation assay does not always accurately reflect the growth-promoting activity of a mitogen; the apparent observed inhibition of incorporation may reflect an effect on nucleoside phosphorylation or uptake rather than the desired effect on cell proliferation. The results further imply that great care must be used in the interpretation of ^3H -thymidine incorporation assays for the studies of growth inhibitors and other types on chemotherapeutic agents.

Carson et al (1988) noted the presence of a low molecular weight factor in ovine follicular fluid that inhibited ^3H -thymidine incorporation into 3T3 cells. This factor may or may not be similar to the ^3H -thymidine incorporation-inhibiting factor in rSCCM. Although we have not clearly defined the role of this factor in spermatogenesis, our recent studies suggest that this factor may potentially play a key role in nucleoside utilization by the germ cells during development (Lamb et al, 1994).

Acknowledgments

We thank Cheryl Gonzales and Nancy Williamson (participants in the Summer Medical and Research Training [SMART] Program for Undergraduate students at Baylor College of Medicine), and Gerald Spotts, Kely Baker, and Sarah Baker for excellent technical assistance. We also thank Dr. Roy G. Smith (Merck, Sharpe and Dohme Research Laboratories) for reading the manuscript.

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