Proc. Natl. Acad. Sci. USA
Vol. 77, No. 8, pp. 4774–4778, August 1980
Cell Biology

Sertoli cells contain a mitogenic polypeptide

(testis/growth factor/cell proliferation/3T3 cells/spermatogenesis)

LARRY A. FEIG*†, ANTHONY R. BELLVÉ*†, NICOLETTE HORBACH ERICKSON‡, AND MICHAEL KLAGSBRUN‡

Departments of *Physiology and ‡Biological Chemistry and †Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02115

Communicated by John R. Pappenheimer, May 8, 1980

ABSTRACT **The seminiferous epithelium of the mouse testis contains a potent mitogen that induces DNA synthesis and cell division in cultures of confluent, quiescent BALB/c 3T3 cells. Homogenates of adult mouse seminiferous tubules, when added to 3T3 cells for 48 hr, produce a dose-dependent increase in DNA synthesis with half-maximal activity at 400 μg of protein per ml. Maximal stimulation causes a 70- to 100-fold increase in [*methyl*-^{3}H]thymidine incorporation into DNA, a labeling of >99% of 3T3 cell nuclei, and a doubling in cell number. The level of mitogenic activity in seminiferous tubules isolated from newborn mice is five times greater than that in tubules of adult mice. Furthermore, prepuberal Sertoli cells contain substantially greater levels of mitogenic activity than any other cell type purified from the seminiferous epithelium. A homogenate of prepuberal Sertoli cells induces half-maximal DNA synthesis at 20 μg of protein per ml. The mitogenic factor is sensitive to proteases and to heating at 100°C for 2 min but not to dithiothreitol. Gel-filtration chromatography reveals that the factor is a polypeptide with a M_r of ≈ 15,500. These observations provide evidence for the presence of a growth factor in the mammalian testis.**

The mammalian testis displays complex patterns of cell proliferation. Primordial germ cells in the early embryo migrate from the yolk sac endoderm to the genital ridges and proliferate rapidly to form the gonocytes that populate the developing seminiferous cords (1). These stem cells, although undergoing growth, cease to divide for about 10 days late in fetal life and early in postnatal development (2). In the mouse and rat, gonocytes reinitiate mitotic cell divisions 4–5 days after birth, the first division yielding primitive type A spermatogonia. Subsequent cell divisions produce a differentiation sequence of type $A_{(1-4)}$, intermediate and type B spermatogonia, and then preleptotene spermatocytes (3, 4). These cells undergo meiosis and finally spermiogenesis, which culminates with the formation of spermatozoa (5). In the adult, active and continuous proliferation of spermatogonia ensures an abundant supply of mature sperm cells.

Testicular somatic cells also undergo unique patterns of proliferation during development. Leydig cells divide rapidly during late fetal life, atrophy in the neonate, but then renew proliferation at early puberty (6). In the adult, Leydig cells rarely divide unless experimentally perturbed (7). Sertoli cells, in contrast, proliferate until early puberty, when they differentiate terminally and never divide again (8, 9).

These varied patterns of cell proliferation suggest mitogenic factor(s) may exist in the testis to regulate cell cycle kinetics. This possibility was examined by testing confluent BALB/c 3T3 cells, which respond to a number of polypeptide mitogens (10–13). Evidence presented in this report demonstrates the existence of a mitogenic polypeptide localized primarily in mouse Sertoli cells.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S.-C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Culture. Mouse BALB/c 3T3 embryo fibroblasts (clone A31) were obtained from C. D. Scher (Harvard Medical School, Boston, MA) and maintained at 37°C in Dulbecco's modified Eagle's medium (Flow Laboratories, McLean, VA) supplemented with glucose (4.5 gm/l), 10% calf serum (Colorado Serum, Denver, CO), penicillin (50 units/ml), and streptomycin sulfate (50 μg/ml; GIBCO).

Preparation of Testis and Seminiferous Tubule Samples. CD-1 mice (Charles River Breeding Laboratories) were used. Adult mice, 10–12 weeks old, were anesthetized and perfused via the thoracic aorta with 10 ml of enriched Krebs–Ringer bicarbonate medium to remove blood from the testes (14). The testes were then excised, decapsulated, minced, and sonicated in phosphate-buffered saline (pH 7.4) at 100 W for 30 sec (Braunsonic, no. 1510). Typically, one testis was sonicated in 4 ml of standard saline, and 20–50 μl of the homogenate was assayed for growth-promoting activity.

Seminiferous tubules were isolated by incubating prepuberal and adult decapsulated testes with collagenase (1.0 mg/ml; Worthington) in the enriched bicarbonate medium under 5% CO_2 at 33°C for 15 min with gentle shaking (15, 16). The dispersed seminiferous tubules were allowed to settle and the supernatant fluid was decanted to remove interstitial and blood elements. The seminiferous tubules were washed in standard saline, homogenized, and then used for the growth-promoting assay. For autoradiography and biochemical characterization, particulate matter was removed from homogenates by centrifugation at 100,000 × g_{av} for 1 hr. All mitogenic activity was retained in the supernatant fraction. Protein content was determined by the method of Lowry *et al.* (17).

Separation of Seminiferous Epithelial Cells. Seminiferous tubules (or cords), prepared as described above, were dissociated to form a cell suspension by incubation with trypsin (0.5 mg/ml) and DNase (2 μg/ml) in enriched Krebs–Ringer bicarbonate medium for 15 min at 33°C. The respective cell types were separated by sedimentation velocity (14, 15). The cells were layered onto a 2–4% gradient of bovine serum albumin in the enriched bicarbonate medium, allowed to settle for 4 hr, and collected. Populations of Sertoli cells (99% pure); primitive type A (90%), type $A_{(1-4)}$ (91%), and type B (76%) spermatogonia; preleptotene spermatocytes (93%); and a pool of leptotene/zygotene spermatocytes (75%) were obtained from prepuberal mouse testes (15, 16). Adult mice provided populations of pachytene spermatocytes (89%), round spermatids (91%) (8), and residual bodies (89%) (14). Cells were identified by differential interference microscopy.

DNA Synthesis. The 3T3 cells in monolayers were trypsinized and suspended at 5 × 10^4 cells per ml in Dulbecco's modified Eagle's medium containing 10% calf serum, and then 200-μl aliquots were placed into 0.3-cm^2 culture wells (Microtest II, Falcon; Bioquest, Cockeysville, MD). After incubation for 7–10 days without a change of medium, the cells became

quiescent. These confluent monolayers, upon addition of 50 μl of experimental sample and 4 μCi/ml of [*methyl*-^{3}H]thymidine [specific activity, 6.7 Ci/mmol (1 Ci = 3.7 × 10^{10} becquerels); New England Nuclear], were incubated for another 40–48 hr. The cells were then fixed in absolute methanol, washed with H_2O, extracted with 5% trichloroacetic acid and dissolved in 0.3 N NaOH (18). Incorporation of [*methyl*-^{3}H]thymidine into DNA was quantified by scintillation counting. Cells also were subjected to autoradiographic analysis (18). Typically, background incorporation of [*methyl*-^{3}H]thymidine in untreated cells was ≈1500 cpm, and only 1% of the nuclei was labeled. Maximal stimulation achieved by adding fresh serum (final concentration 20%, vol/vol) was approximately 100,000 cpm and >95% labeled nuclei.

Cell Division. Confluent 3T3 cells were stimulated at 0 hr and 48 hr with homogenates of either testes or seminiferous tubules. Then the cells were suspended by incubation with 0.25% trypsin and 0.5 mM EDTA in phosphate-buffered saline, Ca^{2+}- and Mg^{2+}-free, and were counted using a Coulter model 2F particle counter (Coulter Electronics, Hialeah, FL).

Characterization of the Mitogenic Factor in Adult Seminiferous Tubules. Sensitivity of the mitogen to protease was tested by incubating the homogenate supernatant fraction in phosphate-buffered saline at 37°C for 4 hr with (*i*) buffer alone; (*ii*) a mixture of trypsin and α-chymotrypsin, each at a concentration of 500 μg/ml (Sigma); or (*iii*) trypsin and chymotrypsin previously inactivated by heating at 100°C for 20 min. After the 4-hr experimental period, trypsin and chymotrypsin were inactivated by adding 2 mg of soybean trypsin inhibitor per ml (Sigma). Other samples were incubated with soybean trypsin inhibitor alone. All samples were assayed for mitogenic activity as outlined above.

Supernatant samples were also exposed for 2 hr at 25°C to 5 mM dithiothreitol in standard saline or to the saline alone. Other aliquots were subjected to 100°C for 2 min. These samples were dialyzed against three changes of 5 mM NH_4HCO_3, lyophilized, and tested for mitogenic activity.

Gel-Filtration Chromatography. Supernatant fractions of a homogenate of adult mouse seminiferous tubules were subjected to gel-filtration chromatography on Bio-Gel A-0.5 (2.5 × 60 cm) equilibrated with 4 M guanidine hydrochloride in 20 mM 2(*N*-morpholino)ethanesulfonic acid, pH 7.6, at 4°C. Samples containing ≈80 mg of protein in 4 ml were applied to the column in buffered guanidine hydrochloride and eluted at a flow rate of 30 ml/hr. Fractions (5 ml) were pooled in groups of three, dialyzed against 20 mM NH_4HCO_3, pH 7.6 (48 hr, four changes), and lyophilized. Each pooled sample was tested for the ability to stimulate DNA synthesis in confluent 3T3 cells.

RESULTS

DNA Synthesis Induced by Testis Homogenate. Testis homogenates stimulated [*methyl*-^{3}H]thymidine incorporation into DNA of confluent, quiescent BALB/c 3T3 cells in a dose-dependent manner. Half-maximal activity occurred at 400 μg of protein per ml (Fig. 1). The maximal response represented 70- to 100-fold stimulation in comparison to unstimulated cells. Homogenates of seminiferous tubules also stimulated DNA synthesis in confluent BALB/c 3T3 cells, generating a dose-response curve similar to that found with testis homogenates (Fig. 1). Autoradiography was used to determine the proportion of 3T3 cells stimulated to synthesize DNA by samples of testis and seminiferous tubules. In these experiments, particulate matter was removed from tissue homogenates by ultracentrifugation. The resulting supernatant fractions exhibited a 2-fold increase in specific activity. These samples also

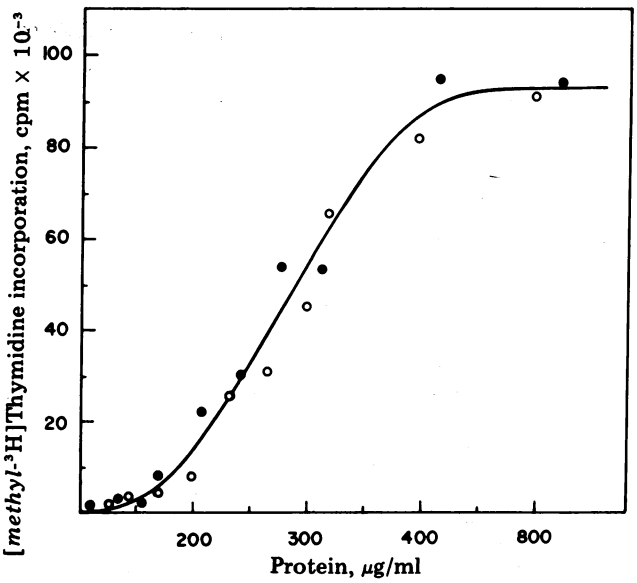

FIG. 1. Dose–response curve for DNA synthesis in BALB/c 3T3 cells stimulated by homogenates of mouse testes and seminiferous tubules. Aliquots of homogenized, perfused testes or seminiferous tubules were added, together with [*methyl*-^{3}H]thymidine (4 μCi/ml), to confluent, quiescent BALB/c 3T3 cells. DNA synthesis was assayed after a 48-hr incubation by quantifying the amount of [*methyl*-^{3}H]-thymidine incorporated into trichloroacetic acid-precipitable material. ○, Testes; ●, seminiferous tubules.

produced equal dose-dependent increases in DNA synthesis, with over 95% of the nuclei labeled when maximal amounts were added (Fig. 2). Since seminiferous tubules constitute >90% of the total testis weight (19), this evidence suggests that the bulk of the testicular growth-promoting activity resides within the seminiferous tubules.

Cell Division Induced by Testes Preparations. Homogenates of testes or seminiferous tubules stimulated cell divisions as well as DNA synthesis in confluent 3T3 cells. These homogenates induced a dose-dependent increase in cell number, with maximal stimulation causing a 5- to 6-fold rise within 7 days (Fig. 3).

Temporal Pattern of Mitogenic Activity During Development. The seminiferous epithelium consists of two classes of cells—epithelial Sertoli cells and differentiating spermatogenic cells. In the mouse, during the first week after birth, the epithelium is comprised solely of the mitotically proliferating spermatogonia (16%) and Sertoli cells (84%) (see ref. 15). However, during the second and third week of development the meiotic spermatocytes and haploid spermatids appear, and these eventually become the dominant cell types (93%) (see ref. 16). Quantifying growth factor activity in seminiferous tubules isolated from mice during postnatal development revealed that the highest activity was present 6 days after birth (Fig. 4). Thereafter, the specific growth factor activity declined substantially. Thus, at postnatal day 20, the level was reduced to one-third and in the adult to one-fifth of that present in the 6-day-old mice. This dilution of activity correlates with the temporal appearance and gradual accumulation of meiotic and postmeiotic germ cells (16). These data suggest that the growth-promoting activity is localized primarily in Sertoli cells and spermatogonia, rather than in meiotic and postmeiotic germ cells.

Mitogenic Activity in Discrete Populations of Seminiferous Epithelial Cells. Preferential localization of the growth factor in particular cells of the seminiferous epithelium can be tested directly. This was accomplished by isolating populations of specific cell types using sedimentation velocity (15, 16). These

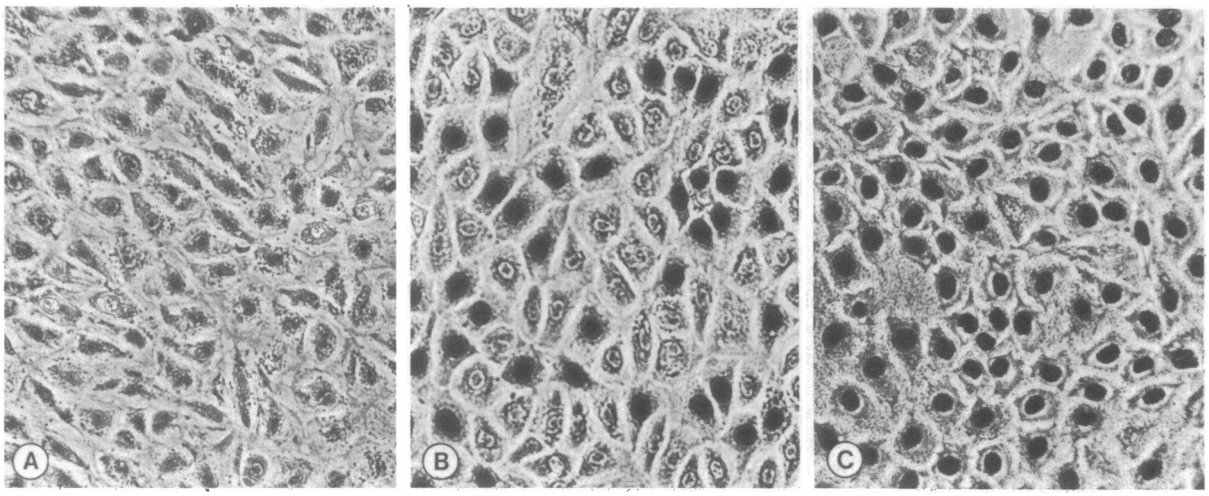

FIG. 2. Stimulation of DNA synthesis in BALB/c 3T3 cells determined by autoradiography. Confluent 3T3 cells were exposed for 48 hr to (100,000 × *g*) supernatant fractions of seminiferous tubule homogenates in the presence of [*methyl*-^{3}H]thymidine (4 μCi/ml). The cultures were fixed and autoradiographed to assess the proportion of cells with labeled nuclei. Similar results were obtained with samples of testis. (*A*) 0 μg of protein per ml (<1% nuclei labeled); (*B*) 160 μg of protein per ml (≈40% nuclei labeled); (*C*) 360 μg of protein per ml (>95% nuclei labeled).

types included Sertoli cells from 6-day-old mice and germ cells at successive stages of differentiation. Homogenates of the respective cell types were tested for their ability to stimulate DNA synthesis in confluent 3T3 cells (Fig. 5). Activity contributed by the few contaminating cells in each purified cell population was subtracted. Clearly, prepuberal Sertoli cells (S6) contained the highest concentration of growth-promoting activity. Half-maximal induction of DNA synthesis in 3T3 cells (50 × 10^3 cpm) occurred in response to 20 μg of protein per ml of a prepuberal Sertoli cell homogenate. Spermatogenic cells with appreciable mitogenic activity were primitive type A spermatogonia and preleptotene spermatocytes, which had a specific activity approximately one-sixth that of prepuberal Sertoli cells. Type $A_{(1-4)}$ and type B spermatogonia; leptotene, zygotene, and pachytene spermatocytes; spermatids; and residual bodies did not contain mitogenic activity (Fig. 5).

Characterization of Seminiferous Tubule-Derived Growth Factor. Seminiferous tubule-derived growth factor is sensitive to protease digestion. Exposing the supernatant fraction of an homogenate to a mixture of trypsin and chymotrypsin for 4 hr at 37°C resulted in >80% loss of activity (Table 1). In control experiments, samples incubated with heat-inactivated trypsin and chymotrypsin, with protease inhibitor alone (data not shown), or with phosphate-buffered saline alone showed no loss of activity. The growth-promoting activity is also heat sensitive. Exposing a sample of seminiferous tubules to 100°C for 2 min

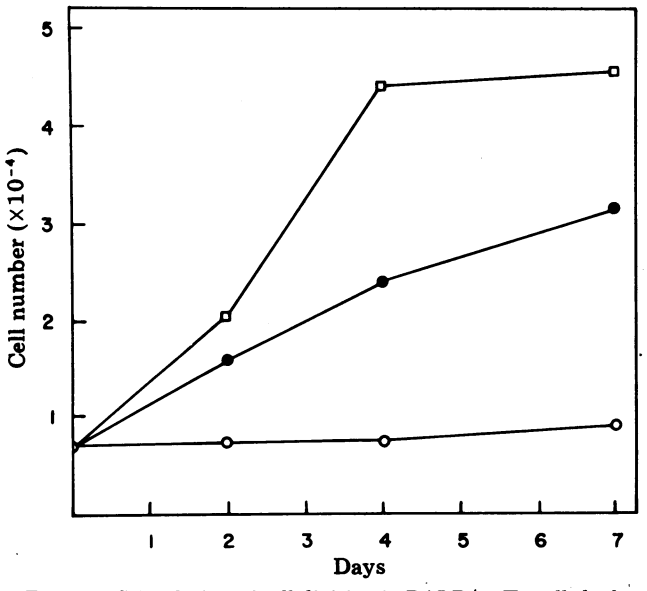

FIG. 3. Stimulation of cell division in BALB/c 3T3 cells by homogenates of seminiferous tubules. Confluent, quiescent 3T3 cells were stimulated at both 0 hr and 48 hr by adding protein from seminiferous tubule homogenates to final concentrations of 0 μg/ml (○), 320 μg/ml (●), or 800 μg/ml (□). Following incubation for 0, 2, 4 and 7 days, the cells were detached with trypsin and cell number was determined. Data represent the mean of two experiments, each performed in duplicate. Similar results were obtained with homogenates of testis.

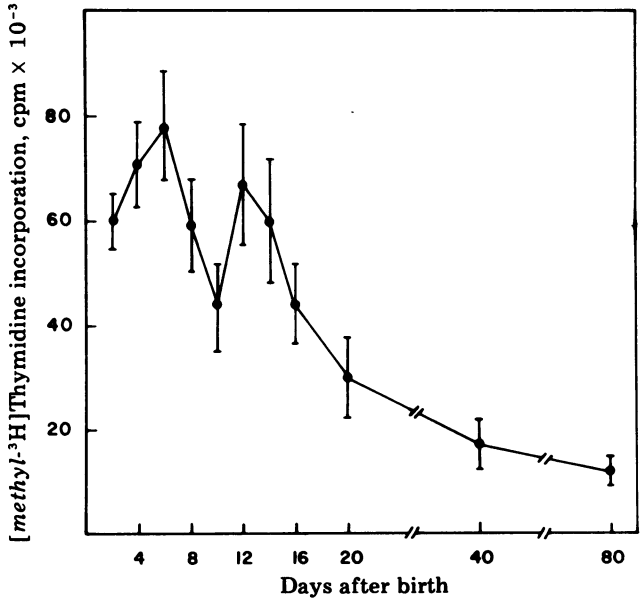

FIG. 4. Mitogenic activity in seminiferous tubules during the postnatal development of mice. Seminiferous tubules were isolated from the testes at various ages after birth. Aliquots (25 μg of protein) of tubule homogenates were added, along with [*methyl*-^{3}H]thymidine (1 μCi), to confluent, quiescent BALB/c 3T3 cells (final volume, 250 μl). Induction of DNA synthesis was assayed, and the results were normalized to an internal standard of maximal stimulation induced by the addition of serum (20% vol/vol). The data represent three independent experiments (mean ± SEM).

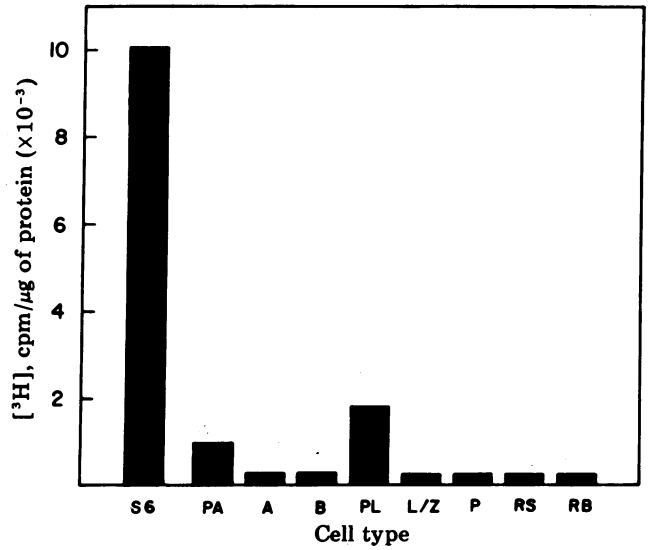

FIG. 5. Growth factor activity in cells isolated from the mouse seminiferous epithelium. Highly purified populations of cells were obtained by velocity sedimentation. The respective cell types were homogenized and aliquots were added to confluent, quiescent BALB/c 3T3 cells (final volume, 250 μl). Induction of DNA synthesis was determined by quantifying the incorporation of [*methyl*-³H]thymidine into trichloroacetic acid-precipitable material. The specific mitogenic activity (cpm/μg of protein) of each cell type was determined from the slope of the dose response curve at half-maximal stimulation. Data are from three independent experiments (SEM < ±15% of mean). Cell types include: Sertoli cells from 6-day-old mice (S6); primitive type A (PA), type $A_{(1-4)}$ (A), and type B (B) spermatogonia; preleptotene (PL), leptotene/zygotene (L/Z), and pachytene (P) spermatocytes; round spermatids (RS); and residual bodies (RB).

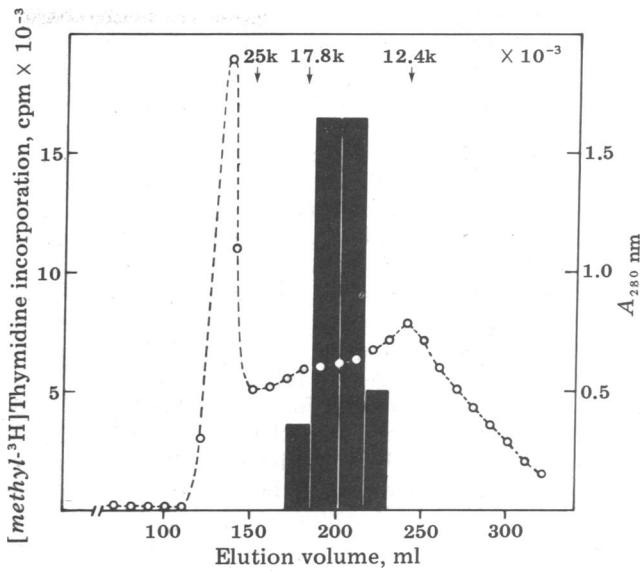

FIG. 6. The supernatant fraction of an adult mouse seminiferous tubule homogenate was chromatographed on Bio-Gel A-0.5 (2.5 × 60 cm). The column was equilibrated with 4 M guanidine·HCl in 20 mM 2(*N*-morpholino)ethanesulfonic acid, pH 7.6. A sample of ≈80 mg of protein was applied and the eluant was collected in 5-ml fractions. Every three successive tubes were pooled and assayed for mitogenic activity. [Standards used were chymotrypsinogen (25,000), myoglobin (17,800) and cytochrome *c* (12,400).] Bars, [*methyl*-³H]thymidine; O---O, absorbance of fraction at 280 nm.

resulted in a total loss of activity. In contrast, dithiothreitol (5 mM) had no adverse effect (Table 1).

During initial experiments, the growth factor activity was eluted in the void volume after chromatography on Sephadex G-100 under nondissociating conditions, indicating the protein has a high molecular weight or exists as part of a multimeric complex. However, when supernatant fractions were chromatographed on Bio-Gel A-0.5 equilibrated with 4 M guanidine·HCl, the aggregate form of the growth factor dissociated to yield a single, symmetrical peak corresponding to a M_r of ≈15,500 (Fig. 6).

Table 1. Characterization of growth factor activity

Treatment	[*methyl*-³H]-Thymidine* incorporation into DNA, cpm
Phosphate-buffered saline	1,580
Tubule supernatant fraction (TS) + saline	55,500
TS + trypsin + α-chymotrypsin	5,400
TS + heat-inactivated trypsin+ α-chymotrypsin	51,050
TS + saline + dialysis	61,400
TS + 5 mM dithiothreitol	65,180
TS + 100°C	2,490

* Seminiferous tubules of adult mice were homogenized and then centrifuged at 100,000 × *g* for 1 hr. Aliquots of the resulting supernatant fraction were incubated at 37°C for 4 hr with a mixture of trypsin and α-chymotrypsin (each at a concentration of 500 μg/ml), a mixture of heat-inactivated trypsin and α-chymotrypsin (each at 500 μg/ml), or phosphate-buffered saline alone. The incubation was terminated by addition of soybean trypsin inhibitor (2 mg/ml). Other samples were exposed at 25°C for 2 hr to the standard saline, to dithiothreitol, or to 100°C for 2 min, then dialyzed (exclusion limit, 6000–8000 daltons), and lyophilized. All samples were tested for their ability to stimulate DNA synthesis in BALB/c 3T3 cells. Data are the average of two experiments, each performed in duplicate.

DISCUSSION

The seminiferous epithelium of the mouse testis contains a polypeptide with a M_r of ≈15,500 that stimulates DNA synthesis and cell division in confluent BALB/c 3T3 cells. This mitogenic factor is resistant to inactivation by dithiothreitol, a sulfhydryl bond-reducing agent that irreversibly inactivates a number of other growth factors, particularly those in blood. These include the platelet-derived growth factor (20), multiplication-stimulating activity (21), and the nonsuppressible insulin-like activity (22). Therefore, the testicular growth factor is probably not one of these serum mitogens.

Mitogenic activity is concentrated primarily in Sertoli cells. This conclusion is based on the following observations. First, purified Sertoli cells contain a greater specific mitogenic activity than any other cell type isolated from the seminiferous epithelium. Second, the level of activity is four times greater in Sertoli cells than in seminiferous tubules from which they are purified. Finally, growth factor activity in the seminiferous tubules declines markedly during postnatal development, concomitant with the decrease in the proportion of Sertoli cells. Although synthesis of the growth factor has not been demonstrated, these findings suggest that the Sertoli cell is the source of mitogenic activity in the seminiferous epithelium.

Sertoli cells are somatic cells that form the seminiferous epithelium within which spermatogenic cells differentiate. Sertoli cells may serve many functions in spermatogenesis, including (*i*) creating a microenvironment necessary for germ-cell differentiation; (*ii*) acting as target cells through which follicle-stimulating hormone and testosterone influence spermatogenesis; and (*iii*) selectively translocating germ cells from the peripheral to the central compartment of the seminiferous epithelium (23). It is tempting to speculate that the Sertoli

cell-derived growth factor plays a role in the regulation of spermatogenesis. An exciting possibility is that this factor mediates the active proliferation of spermatogonia. Other potential target cells include preleptotene spermatocytes and interstitial cells.

Factors regulating the proliferation of spermatogonia are poorly understood. Although some evidence suggests the existence of a spermatogonial chalone (24), an inhibitor of spermatogonia proliferation, these observations have not been substantiated (25). In contrast to a chalone, the Sertoli cell-derived growth factor provides a mechanism for a positive regulation of spermatogenesis. It is plausible to speculate that primitive type A spermatogonia and preleptotene spermatocytes are targets for the growth factor, since both cell types contain some mitogenic activity. For instance, the growth factor may be responsible for the onset of DNA synthesis and cell proliferation that occurs in primitive type A spermatogonia when prepuberal spermatogenesis begins. Similarly, the mitogen may stimulate DNA synthesis in preleptotene spermatocytes in order to synchronize cells within syncytia for entry into meiotic prophase and subsequent reduction divisions.

Cells of the interstitial compartment represent another possible target for the growth factor. These cells rarely divide in normal adult animals (6, 19), and yet hyperplasia occurs in response to certain experimental perturbations. Interstitial cell hyperplasia occurs in the cryptorchid testes even in hypophysectomized rats in which gonadotropins are absent (26). Similarly, the inhibition of spermatogenesis by testicular implants of androgen antagonists results in localized hyperplasia of Leydig cells (7). In both cases the ensuing cell proliferation could be due to the local release of the Sertoli cell-derived growth factor.

Mitogenic factors are considered to play a role in the proliferation of other cell types. Erythropoietin regulates erythroblast proliferation (27), whereas the local release of a growth factor from platelets may stimulate cellular division in the arterial wall during wound healing (20). The Sertoli cell-derived growth factor may induce or modulate cell proliferation in the testis and, therefore, be of fundamental significance to mammalian spermatogenesis.

The authors extend their appreciation to Steven Borack for the photographic reproductions and to Mrs. Mary Forte for typing. The research was funded by National Institutes of Health Grants HD-12600, HD-06916, and CA-21763, with further support provided by National Institute of Child Health and Human Development Centers Grant HD-06645 and Rockefeller Foundation Grant RF 65040. L. F. was the recipient of a National Institute of Child Health and Human Development Predoctoral Fellowship, HD-07130, and a Hoechst-Roussel Fellowship. A preliminary report of this research has been presented to the American Society for Cell Biology, Toronto, Canada (28).

1. Clark, J. M. & Eddy, E. M. (1975) *Dev. Biol.* **47,** 136–155.
2. Franchi, L. L. & Mandl, A. M. (1964) *J. Embryol. Exp. Morphol.* **12,** 289–308.
3. Huckins, C. (1971) *Cell Tissue Kinet.* **4,** 139–154.
4. Huckins, C. & Oakberg, E. F. (1978) *Anat. Rec.* **192,** 519–528.
5. Bellvé, A. R. (1979) in *Oxford Reviews of Reproductive Biology,* ed. Finn, C. A. (Oxford University Clarendon Press, Oxford), Vol. 1, pp. 159–261.
6. Baillie, A. H. (1961) *J. Anat.* **95,** 357–370.
7. Aoki, A. & Fawcett, D. W. (1978) *Biol. Reprod.* **19,** 144–158.
8. Steinberger, A. & Steinberger, E. (1971) *Biol. Reprod.* **4,** 84–87.
9. Nagy, F. (1972) *J. Reprod. Fert.* **28,** 389–395.
10. Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.,* **48,** 193–216.
11. Gospodarowicz, D. & Moran, A. (1974) *Proc. Natl. Acad. Sci USA* **71,** 4584–4588.
12. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1809–1813.
13. Klagsbrun, M. (1978) *Proc. Natl. Acad Sci. USA* **75,** 5057–5061.
14. Romrell, L. J., Bellvé, A. R. & Fawcett, D. W. (1976) *Dev. Biol.* **49,** 119–131.
15. Bellvé, A. R., Cavicchia, J. C., Millette, C. F., O'Brien, D. A. Bhatnagar, Y. M. & Dym, M. (1977) *J. Cell Biol.* **74,** 68–85.
16. Bellvé, A. R., Millette, C. F., Bhatnagar, Y. M. & O'Brien, D. A. (1977) *J. Histochem. Cytochem.* **25,** 480–484.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Klagsbrun, M., Langer, R., Levinson, R., Smith, S. & Lillehei, C. (1977) *Exp. Cell Res.* **105,** 99–108.
19. Reddy, K. J. & Svoboda, D. J. (1967) *Arch. Pathol.* **84,** 376–392.
20. Ross, R. & Vogel, A. (1978) *Cell* **14,** 203–210.
21. Temin, H. M., Smith, G. L. & Dulak, N. C. (1974) in *Control of Proliferation in Animal Cells,* eds. Clarkson, B. & Baserga, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 1, pp. 19–26.
22. Oelz, O., Froesch, E. R., Bunzli, H. F., Humbel, R. E. & Ritschard, W. J. (1972) in *Handbook of Physiology,* eds. Greep, R. O. & Astwood, E. B. (Waverly, Baltimore, MD), Vol. 1, pp. 685–702.
23. Fawcett, D. W. (1977) in *Frontiers in Reproduction and Fertility Control,* eds. Greep, R. O. & Koblinsky, M. A. (M.I.T. Press, Cambridge, MA), Part 2, pp. 302–320.
24. Clermont, Y. & Mauger, A. (1976) *Cell Tissue Kinet.* **9,** 99–104.
25. Cunningham, G. R. & Huckins, C. (1979) *Cell Tissue Kinet.* **12,** 81–90.
26. Iturriza, F. C. & Irusta, O. (1979) *Acta Physiol. Lat. Am.* **19,** 236–242.
27. Adamson, J. W. & Brown, J. E. (1978) in *Molecular Control of Proliferation and Differentiation,* eds. Papaconstantinou, J. & Rutter, W. J. (Academic, New York), pp. 161–179.
28. Feig, L. A., Bellvé, A. R., Horbach, N. & Klagsbrun, M. (1979) *J. Cell Biol.* **83,** 220a.