A novel collagen-glycosaminoglycan (C-GAG) substrate was developed to overcome the optical opacity of a HATF nitrocellulose substrate and to provide a more physiological permeable substrate for cocultured Sertoli and spermatogenic cells. Cocultures were prepared on optically transparent C-GAG discs attached to a polyester mesh to facilitate handling. Sertoli cells displayed a cuboidal-to-columnar shape; a large number of spermatogonia and primary spermatocytes connected by intercellular bridges were associated with basolateral and apical surfaces of Sertoli cells up to 12 days after plating. Rat Sertoli-spermatogenic cell cocultures have been used for testing the effect of toxicants on rat spermatogenesis in vitro. In our initial studies, we tested the effects of the toxicant gossypol on spermatogenic cells cocultured with Sertoli cells on non-permeable (plastic) and permeable substrates (HATF nitrocellulose) under both standard culture conditions and during perifusion after achieving a continuous electrical-resistant cell monolayer. A selective mitochondrial structural damage was observed in spermatogenic cells (spermatogonia and spermatocytes) but not in the coexisting Sertoli cells. This damage was time- (15-60 min) and dose-dependent (0.1-10μM) and developed more rapidly under perifusion conditions. Similar mitochondrial damage was reported in the intact animal but required higher concentrations (mg) and longer administration time (months) for detection. Studies are in progress to evaluate the effect of additional toxic chemical agents on functional properties of Sertoli and spermatogenic cells in cocultures prepared on various classes of C-GAG substrates.
INTRODUCTION

Spermatogenesis in vitro is a valuable approach for the study of bioregulatory molecules of the spermatogenic process and for testing selective effects of toxicants on proliferating and differentiating spermatogenic cells. The development of suitable in vitro experimental conditions depends on the maintenance of two unique biological characteristics of the spermatogenic process: the spermatogenic stage-dependent functional cycle of Sertoli cells (Kierszenbaum, 1974; Parvinen, 1982; Shabanowitz et al., 1986) and the clonal-like organization of spermatogenic cells resulting from cytoplasmic bridges linking cells of the same progeny (Dym and Fawcett, 1971; Erickson, 1972).

Sertoli cells are essential for the viability and differentiation of spermatogenic cells in culture (Tres and Kierszenbaum, 1983). Additional conditions required for achieving long-term viability and differentiation of spermatogenic cells in vitro include: (1) minimal disruption of the structural relationship that Sertoli and spermatogenic cells maintain in vivo during enzymatic dissociation of the seminiferous epithelium; (2) cell plating at maximum cell density (~3 x 10^6 cells/ml) to maintain Sertoli cells in a contact inhibited state, and (3) frequent replenishment of a serum-free, hormone/growth factor-supplemented culture medium and removal of waste metabolic products from the contiguous cellular environment (Kierszenbaum and Tres, 1987). In the intact testis, metabolic products are released into the seminiferous tubular lumen and into lymphatic and blood vessels surrounding the seminiferous tubules. An automated cell perifusion system was developed to deliver successive pulses of hormones and growth factors to rat Sertoli-spermatogenic cell cocultures prepared on a permeable substrate while removing waste metabolic products (Kierszenbaum and Tres, 1987). More recently, we have used defined substrates synthesized from purified components of the extracellular matrix (ECM) (Tres et al., 1991, 1992). These substrates are optically transparent, permeable and formed from a colloidal co-precipitate of purified collagen type I and glycosaminoglycans crosslinked to form an insoluble analog of the ECM.

In this paper, we report the growth properties of Sertoli and spermatogenic cells on collagen-glycosaminoglycan (C-GAG) permeable substrates for further evaluation of bioregulatory molecules and toxicants on spermatogenesis in vitro. In addition, we report time- and dose-dependent effects of the toxicant gossypol on spermatogenic cells cultured with Sertoli cells prepared on non-permeable (plastic) and permeable (HATF nitrocellulose) substrates. These studies are the initial step toward the evaluation of several toxicants using Sertoli-spermatogenic cell cocultures prepared on C-GAG.

METHODS

Preparation of Rat Sertoli-spermatogenic Cell Cocultures
Cocultures were prepared on plastic, nitrocellulose and C-GAG substrates using 20-22 day old pubertal rats (Tres and Kierszenbaum, 1983) and maintained in serum-free culture medium