BIOLOGY OF REPRODUCTION 35, 1025–1035 (1986)

Stage-Specific Synthesis and Fucosylation of Plasma Membrane Proteins by Mouse Pachytene Spermatocytes and Round Spermatids in Culture[1]

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ABSTRACT

Little is known about the ability of mammalian spermatogenic cells to synthesize plasma membrane components in the presence or absence of Sertoli cells. In this study, purified populations (>90%) of pachytene spermatocytes or round spermatids were isolated by unit gravity sedimentation and cultured for 20–24 h in the presence of [^{35}S]methionine or [^{3}H]fucose. Cell viabilities remained over 90% during the course of these experiments. Plasma membranes were purified from these cells and analyzed by two-dimensional gel electrophoresis. Qualitatively, the same plasma membrane proteins were synthesized by both cell types with the exception of the major Concanavalin A-binding glycoprotein, p151; the synthesis of p151 is greatly diminished or inhibited after meiosis. [^{3}H]Fucose was incorporated into at least 6 common glycoproteins of both cells. Eight components fucosylated with molecular weights from 35,000 to 120,000 were specific to pachytene spermatocyte membranes. One fast-migrating fucosylated component may represent an uncharacterized lipid whose synthesis is terminated after meiosis. Round spermatids specifically fucosylated two components with molecular weights of 45,000 and 80,000. These results demonstrate the viability of germ cells of the male mouse in short-term culture and show that they are capable of synthesizing and fucosylating plasma membrane components in the absence of Sertoli cells.

INTRODUCTION

Relatively little is known about the control of mammalian spermatogenesis at the level of the germ cell. Partly, this lack of knowledge exists because spermatogenesis is a complex system of differentiation: there are intricate relationships between germ cells and Sertoli cells, and germ cells develop as a synctium of cells at a given stage of maturation (Fawcett et al., 1959; Russell, 1980). Spermatogenesis is also poorly understood because, unlike other systems of differentiation, conditions are not currently available that allow extensive differentiation of isolated spermatogenic cells in vitro. For this reason, most studies of mammalian male germ cells have been descriptive rather than analytical, and most in vitro studies have been limited to periods of less than 10 h. Assessing culture conditions for these cells is difficult because mammalian spermatocytes do not complete meiosis in vitro and spermatids remain viable for only a short period of time in culture. In addition, no hormones are known to act directly on spermatocytes or spermatids to alter any of their functions (Fritz, 1978).

To investigate the molecular interactions at the cell surfaces of male germ cells and Sertoli cells and to characterize the biochemical changes associated with the production of the plasma membrane of a mature spermatozoon, our research focused on the properties of germ cell plasma membranes. The plasma membranes from spermatocytes and spermatids were isolated and analyzed by two-dimensional electrophoresis (Millette et al., 1980; Millette and Moulding, 1981a,b). Cell surface molecules were characterized by vectoral iodination, by immuno-

Accepted April 16, 1986.
Received January 27, 1986.
[1] Funding for this study was provided by NIH grants HD-11267 and HD-15269.
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chemical methods, and by antibody and lectin-blotting procedures (Millette and Moulding, 1981b; Millette and Scott, 1984; O'Brien and Millette, 1984). In toto, the results from these experiments demonstrated minor but reproducible differences in the plasma membranes from pachytene spermatocytes and round spermatids.

For a variety of reasons, we suspected that other more distinct differences existed between the plasma membranes of pachytene spermatocytes and round spermatids. First, pachytene spermatocytes have been shown to have a greater affinity for binding of Sertoli cells in vitro than do round spermatids (Enders and Millette, 1984). Second, since the haploid round spermatid is beginning the final phase of differentiation to become a mature spermatozoon, extensive remodeling of the cell surface is likely to start at this stage. Third, late germ cells contain high levels of dolichol, an isoprenoid involved in the synthesis of asparagine-linked carbohydrate side-chains (James and Kandutsch, 1980; Potter et al., 1981).

We have begun to examine the biosynthesis of proteins and glycoproteins by adult mouse spermatogenic cells in the absence of Sertoli cells with the idea that differences in the synthesis of particular proteins or glycoproteins might reflect more accurately the changes associated with the physiological properties of these germ cells. To conduct these studies, it was necessary to isolate mouse pachytene spermatocytes and round spermatids for short-term primary cell culture. Recently, we showed that early round spermatids cultured in the manner used in this study are capable of forming flagella in vitro, indicating that these cells are indeed capable of some aspects of their normal physiological differentiation in the absence of Sertoli cells (Gerton and Millette, 1984). Here, we demonstrate that spermatogenic cells from the adult mouse remain viable for at least 24 h in culture. These isolated cells are capable of synthesizing proteins and, in particular, synthesize an essentially normal array of plasma membrane proteins. Pachytene spermatocytes and round spermatids also fucosylate glycoproteins; some of these fucosylated components are specific to individual spermatogenic cell types.

MATERIALS AND METHODS

Animals and Cell Preparation

Adult CD-1 mice aged 60–120 days obtained from Charles River Laboratories (Wilmington, MA) or adult TAC:(SW)fBR mice of the same age from Taconic Farms, Inc. (Germantown, NY) were used for these studies. Similar results were obtained with mice of both strains.

Testicular cells were isolated by procedures previously described (Bellvé et al., 1977; Romrell et al., 1976). Testes were removed, decapsulated, and immediately placed into sterile enriched Krebs-Ringer bicarbonate buffer (EKRB) as formulated by Romrell et al. (1976). With the exception of the cell-separation step, all subsequent procedures utilized sterile technique. Seminiferous cell suspensions were prepared by use of sequential incubations in 0.5–0.75 mg/ml collagenase and 0.5 mg/ml trypsin as described previously (Bellvé et al., 1977). Purified populations (>90% pure) of pachytene spermatocytes and round spermatids were obtained by unit gravity sedimentation in linear 2%–4% wt/vol gradients of bovine serum albumin (BSA, Fraction V, US Biochemical Corp., Cleveland, OH). Logistically, it was not possible to perform these cell separations in a sterile environment. To minimize microbial contamination, all glassware and tubing were autoclaved before use, and the BSA was sterilized by filtration (millipore IVAA0103F prefilter followed by SVG01015 sterilizing filter, Millipore Corp., Bedford, MA).

Cell Culture

Purified pachytene spermatocytes or purified round spermatids were washed twice in sterile EKRB prior to stationary cell culture. Cells were cultured in Eagle's minimal essential medium supplemented with 10% dialyzed fetal bovine serum (GIBCO Laboratories, Grand Island, NY), 6 mM sodium lactate, 1 mM sodium pyruvate, 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin. For the metabolic labeling of proteins, cells were cultured in the above medium lacking methionine with [^{35}S]-methionine (Amersham, Arlington Heights, IL) added at the indicated concentration. For the labeling of fucose-containing glycoconjugates, cells were cultured in the above medium containing L-[6-^{3}H]fucose (Amersham) at the concentrations indicated in the figure legends. Plasma membranes were purified from these cells by the method of Millette et al. (1980). This procedure involves a brief hypotonic shock, followed by controlled cell homogenization to obtain supernatants enriched in surface membranes. These supernatants are then further purified by sucrose den-

sity gradient centrifugation. Purity of the resultant plasma membrane fractions has been assessed both morphologically, at the ultrastructural level, and biochemically, using surface-bound lectin markers as well as a variety of enzymatic markers for different intracellular compartments (Millette et al., 1980).

Microscopy

Cell viabilities were determined by trypan blue exclusion. Cell morphology and purity were assayed by Nomarski differential interference contrast microscopy (Romrell et al., 1976). For transmission electron microscopy, the cultured cells were washed twice in EKRB. Pellets from the second wash were fixed for 30 min at room temperature in 2.0% glutaraldehyde in 0.1 M sodium phosphate, pH 7.4, and postfixed with 1.0% osmium tetraoxide in 0.1 M sodium phosphate, pH 7.4, for 1 h, also at room temperature. Samples were dehydrated in a graded series of ethanols and embedded in Epon-Araldite. Thin sections were cut and stained with aqueous uranyl acetate and lead citrate according to standard methods. Sections were studied on a Phillips EM300 instrument using 60 kV.

Electrophoretic Procedures

Samples were prepared and analyzed by two-dimensional gel electrophoresis according to the procedure of O'Farrell (1975). Dithiothreitol was substituted for mercaptoethanol in the lysis buffer to achieve isoelectric fosucing pH gradients that were more linear and produced more basic pH values (Righetti et al., 1982).

Cells were silver-stained for protein using a modification of the procedures of Morrissey (1981) and Wray et al. (1981). After electrophoresis was terminated, gels were fixed for over 2 h in 50% methanol containing 1 μl/ml of 37% formaldehyde. The gels were then gently agitated in 5 μg/ml dithiothreitol in water. At the end of 30 min, this solution was replaced with 0.1% AgNO in water, and agitation was continued for another 30 min. The gels were then rinsed with two changes of deionized water and one change of developer (3% sodium carbonate containing 0.5 μl/ml of 37% formaldehyde). Fresh developer was then added, and staining was allowed to develop until the background began to darken. Staining was stopped by the addition of 2.3 M citric acid (5 ml per 100 ml of developer). This procedure routinely resulted in high-contrast, low-background staining of proteins. The gels were immediately photographed wih an MP-4 Polaroid camera and Type 55 film.

To detect [^{35}S]methionine- or [^{3}H]fucose-labeled components, silver-stained gels were completely destained by soaking in film-strength Kodak Rapid-Fix. The gels were then extensively rinsed with deionized water and treated for autofluorography with 1 M sodium salicylate (Chamberlain, 1979). Kodak X-OMAT AR film was exposed to the dried gels at −70°C and processed according to the manufacturer's instructions.

TCA Precipitation

Mixed spermatogenic cells cultured in the presence of radioisotopes were harvested, washed twice with EKRB, and then mixed with trichloroacetic acid (TCA) so that the final concentration was 10% TCA. Precipitates were either trapped on glass filters or collected by centrifugation. The precipitates were then washed with 10% TCA. Glass fiber filters were washed with 95% ethanol, dried, added to a vial containing 4 ml of Ultrafluor (National Diagnostics, Parsippany, NJ), and counted directly in a liquid scintillation counter. Precipitates recovered by centrifugation were dissolved in 100 μl of 0.1 M NaOH, added to 4 ml of Ultrafluor, and counted. Both procedures yielded similar results.

RESULTS

Viability and Morphology of Cultured Spermatogenic Cells

Mixed spermatogenic cells obtained after collagenase and trypsin treatment of seminiferous tubules consist primarily of late pachytene spermatocytes, round spermatids, condensing spermatids, and residual bodies with minor numbers of somatic cells such as Sertoli and Leydig cells (Bellvé et al., 1977). With the culture conditions used in this study, contaminating somatic cells attached to the culture dish plastic, but germ cells did not. The spermatogenic cells could then be recovered without the use of proteases after suspension in the culture medium by gentle tapping and agitation of the culture vessel.

The viabilities of cultured germ cells were assayed by trypan blue exclusion. Initial viabilities were always in excess of 95% and, at cell densities less than 2.2×10^6 cells/cm^2, remained above 90% over 24 h (Fig. 1). Viabilities of germ cells remained high (>85%) after 48 h at these cell densities. An increase

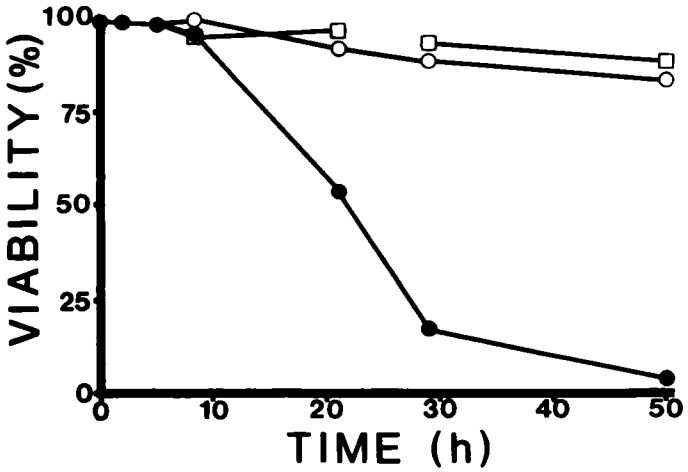

FIG. 1. Viability of cultured mouse spermatogenic cells as a function of time and cell density. *Open squares*, 7.3 × 10^5 cells/cm^2; *open circles*, 2.2 × 10^6 cells/cm^2; *closed circles*, 6.6 × 10^6 cells/cm^2. Initial cell viability was 97.4%. To ensure high cell viabilities for the studies reported here, isolated pachytene spermatocytes and round spermatids were cultured at cell densities less than 1.1 × 10^5 cells/cm^2 and for periods no longer than 24 h.

in the medium acidity was noted at the higher cell densities and, microscopically, we found that cells had settled to form multiple layers. Presumably cell death was accelerated because the cells in the bottom layers did not have adequate access to nutrients and oxygen in the medium.

Cultured germ cells retain normal morphology. Cultured germ cells were examined by electron microscopy at varying times from 0 h to 48 h to determine the degree to which their morphological characteristics resembled cells in situ. Examples of isolated, cultured pachytene spermatocytes and round spermatids are shown in Figure 2. All cell populations examined exhibited excellent preservation of normal intracellular morphology. Pachytene spermatocytes, for example, contained synaptonemal complexes and vacuolated mitochondria typical of these cells before removal from the seminiferous epithelium. Round spermatids exhibited well-defined acrosomal granules and vesicles at varying stages of differentiation. The Golgi complexes of spermatids as well as the more peripheral intracellular localization of mitochondria were also indistinguishable from the normal in situ appearance. Both spermatocytes and spermatids occasionally exhibited remnants of the intracellular bridges that normally connect spermatogenic cells at similar stages of differentiation within the seminiferous tubule. One example of such a remnant is shown in Figure 2A.

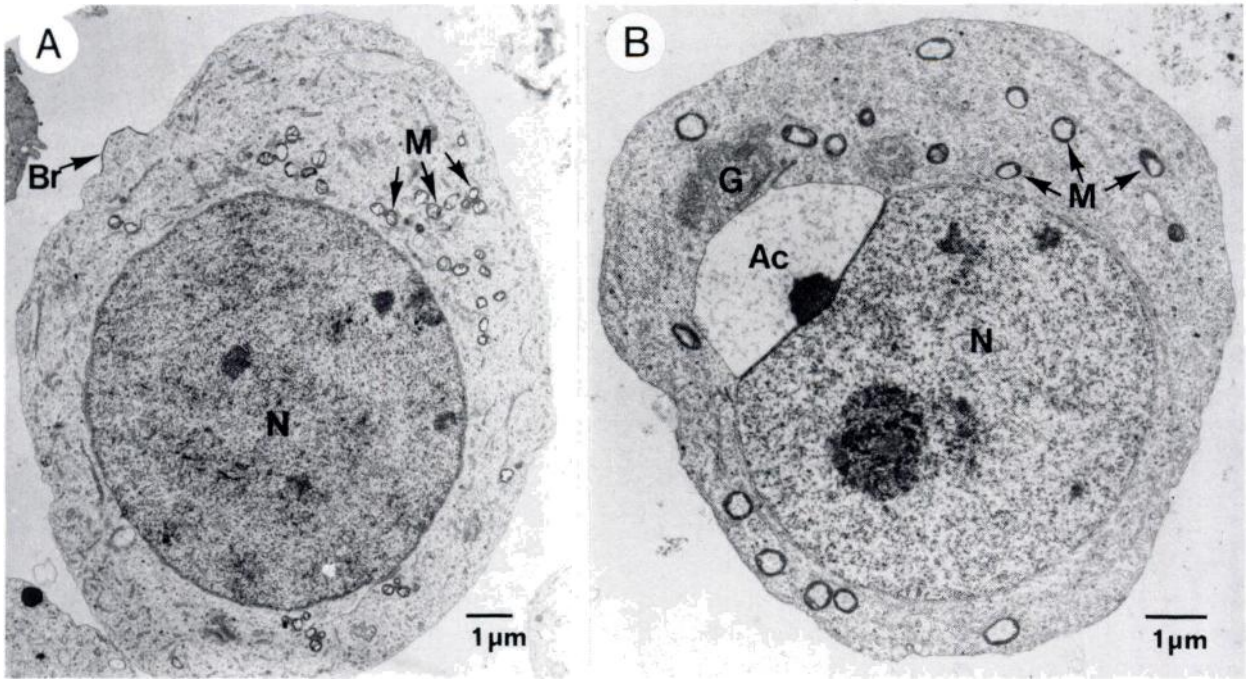

FIG. 2. Ultrastructural appearance of cultured mouse spermatogenic cells. As another indication of general cell viability during culture, isolated populations of pachytene spermatocytes (*A*) and round spermatids (*B*) were examined with the electron microscope. Representative examples are shown here. Cultured cells retained their normal in situ morphology. The individual cells shown were removed from culture at 26 h. (*Ac*) = Acrosome; (*Br*) = remnant of intercellular bridge; (*G*) = Golgi complex; (*M*) = mitochondria; (*N*) = nucleus.

Germ Cells Synthesize a Variety of Proteins

Mixed spermatogenic cells incorporated [^{35}S]-methionine into TCA-precipitable macromolecules. Over a 25-h period, incorporation approached linearity and was approximately proportional to the concentration of the isotope in the culture medium (Table 1).

Populations of pachytene spermatocytes (90.2% pure) and round spermatids (91.0% pure) were prepared by unit gravity sedimentation and cultured for 20 h in the presence of 35 μCi/ml [^{35}S]methionine. The proteins of whole cells were separated by two-dimensional gel electrophoresis and analyzed by silver-staining and autofluorography. Each cell type synthesized over 200 proteins (data not shown). The proteins synthesized were generally represented by silver-stained components. When the patterns generated by proteins of pachytene spermatocytes and round spermatids were compared, very few qualitative differences were detected, but the relative staining intensities of some proteins differed for the two cell types.

Plasma Membrane Proteins are Synthesized by Cultured Germ Cells

Plasma membranes were isolated from [^{35}S]-methionine-labeled pachytene spermatocytes and round spermatids and were analyzed by two-dimensional gel electrophoresis. As was demonstrated earlier (Millette and Moulding, 1981a), silver-stained gels of plasma membrane proteins were qualitatively similar to the staining patterns obtained with Coomassie blue. Very minor differences were seen between the silver-stained patterns of pachytene spermatocytes and round spermatid plasma membrane proteins (Fig. 3a,b).

The autofluorograms and silver-staining patterns of two-dimensional gels of plasma membrane proteins from cultured spermatogenic cells were nearly identical to the silver-staining patterns of membrane proteins from noncultured cells. These results also correlated with the results of Millette and Moulding (1981a), who compared the Coomassie-blue-staining patterns from cultured and noncultured mixed seminiferous cells. Five plasma membrane proteins were described in that paper that were found only after 8 h of culture. In the study presented here, we looked at purified populations of spermatogenic cells after 20 h of culture and found four of those previously described proteins. Two of these components, c1 and c2, were readily detected in plasma membranes from both pachytene spermatocytes and round spermatids (Fig. 3). In spermatocytes, c1 and c2 appear to be synthesized at approximately the same rate, whereas in spermatids, c2 is synthesized at a lower rate. Components c3 and c4 were also detected in both membrane preparations. Component c5, initially described by Millette and Moulding (1981a), was not detected by either silver staining or autofluorography. In the region of the gel where c5 should have been found were two proteins of interest: a very acidic protein, x, was found to be synthesized only by pachytene spermatocytes; and a smaller, slightly more basic protein, y, was found only in preparations of round spermatids (Fig. 3).

Cultured Round Spermatids Synthesize Small Amounts of Protein p151

When pachytene spermatocytes were examined, essentially all the major plasma membrane proteins detected by silver-staining were synthesized by these

TABLE 1. Cell viability and incorporation of [^{35}S]methionine.*

	Time			
	3 hours	6 hours	9 hours	25 hours
Viability	98.2 ± 0.6%	96.8 ± 0.5%	96.4 ± 1.3%	92.3 ± 1.3%
Incorporation (dpm/10^6 cells)				
25 μCi/ml	2.25×10^6	3.98×10^6	5.15×10^6	11.80×10^6
50 μCi/ml	4.15×10^6	7.12×10^6	8.95×10^6	23.60×10^6
100 μCi/ml	7.58×10^6	13.62×10^6	16.88×10^6	38.76×10^6

*2.5×10^6 seminiferous cells were cultured in 0.2 ml of medium containing the specified concentration of [^{35}S]methionine. At the indicated time points, the contents of wells were removed and macromolecules were precipitated with trichloroacetic acid as described in the text. Each value is the average of triplicate points.

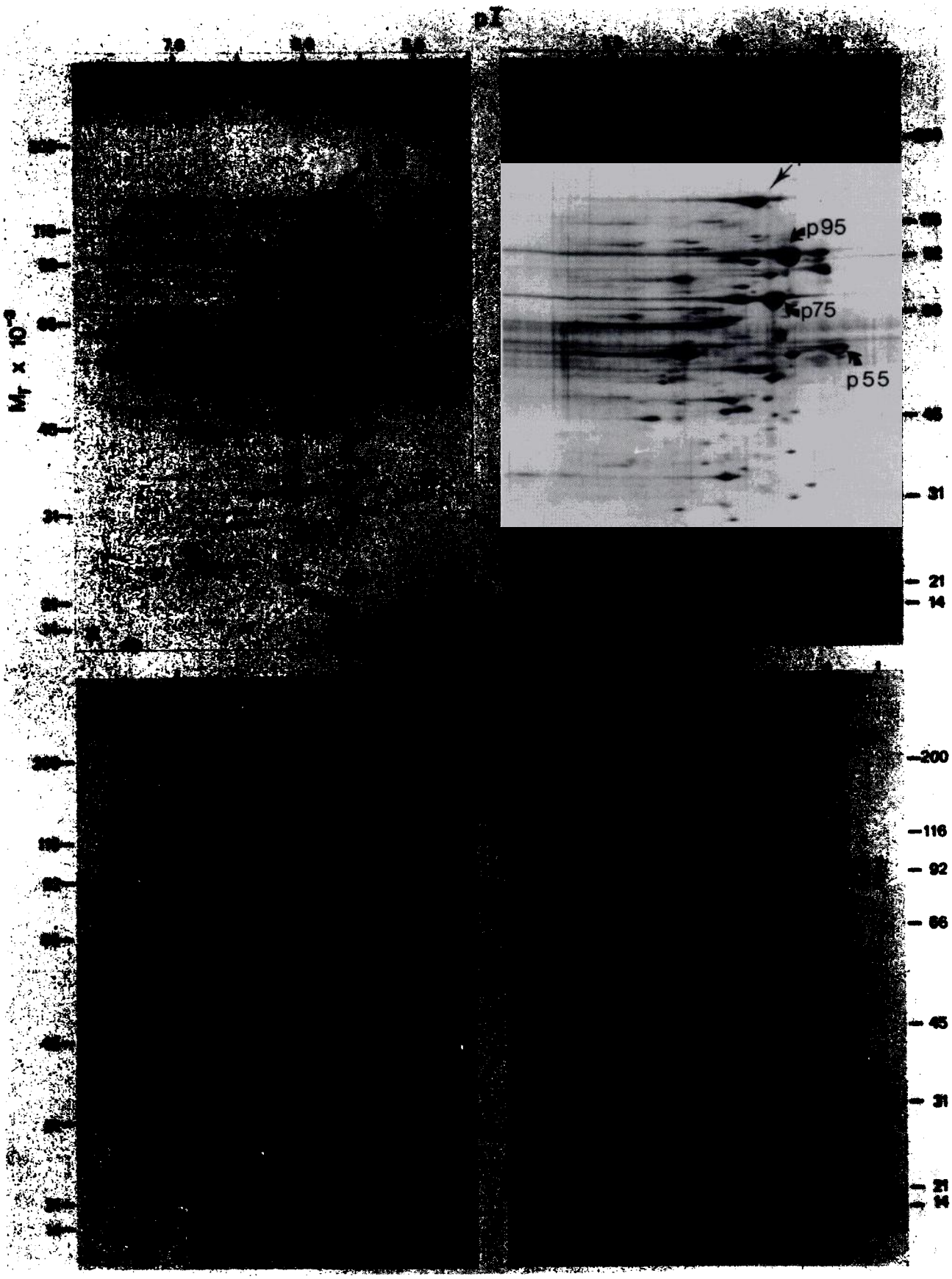

FIG. 3. Analysis of plasma membrane polypeptides synthesized in culture by isolated spermatogenic cells. Pachytene spermatocytes (90.2%) or round spermatids (91.0%) were cultured for 20.5 h at 33°C in 35 μCi/ml [^{35}S]methionine. Plasma membranes were isolated from these cells and analyzed by two-dimensional gel electrophoresis (second dimension gradient gel of 7–12% polyacrylamide). (*A* and *B*) Silver-staining patterns of plasma membrane proteins isolated from cultured pachytene spermatocytes and round spermatids. (*C* and *D*) Autofluorograms of the gels in *A* and *B*, respectively, showing incorporation of [^{35}S]methionine into plasma membrane proteins. *p151*, *p95*, *p75*, and *p55* are major protein-staining components of these plasma membranes. *c1–c4* represent polypeptides previously recognized that undergo alterations concomitant with initial cell isolation and subsequent culture (Millette and Moulding, 1981a). Proteins *x* and *y* represent minor constitutents synthesized only by pachytene spermatocytes and round spermatids, respectively.

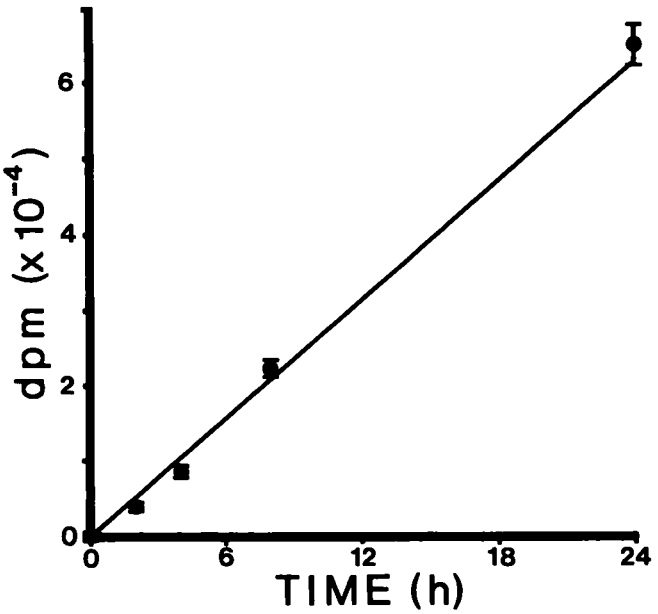

FIG. 4. Incorporation of [6-^{3}H]-L-fucose by isolated germ cells as a function of time. Mixed seminiferous cells were cultured in the presence of 10 μCi/ml of L-[6-^{3}H]fucose. At the indicated time, cells were harvested and the total cell incorporation was assayed as described in the text. Each *point* represents the average (± one standard deviation) of triplicate determinations.

cells. In contrast, a very intensely silver-stained protein of both the pachytene spermatocyte and round spermatid plasma membranes was synthesized to a negligible extent by the latter cell type (Figs. 3b,d). The synthesis of this 151,000-molecular-weight protein (p151) is apparently terminated after meiosis. Its rate of degradation must be slow since this protein was still present in the round spermatid plasma membrane after 24 h of culture. Another example of the persistence of p151 in germ cell plasma membranes is that in a previous study, p151 was also found in plasma membranes prepared from residual bodies (Millette and Moulding, 1981a).

Stage-Specific Fucosylation of Plasma Membrane Components Occurs in Culture

Adult mouse spermatogenic cells were cultured for 20–24 h in the presence of 40–60 μCi/ml of L-[6-^{3}H]fucose. Incorporation into TCA-precipitable material was linear over a 24-h period (Fig. 4). Purified populations of pachytene spermatocytes and round spermatids were cultured in the presence of [6-^{3}H]-L-fucose, and the radioactive products synthesized by these two cell types were analyzed by two-dimensional gel electrophoresis. Both cell types incorporated fucose into more than 15 components (Fig. 5). Many of these components did not silver stain for protein or incorporate detectable amounts of [^{35}S]methionine and may therefore represent minor constituents of the plasma membrane. Of these, at least six, including a component migrating at the position of p151, are fucosylated by both cell types. Eight components with molecular weights from 35,000 to 120,000 are specific to pachytene spermatocyte plasma membranes. One component wih an isoelectric point from 6.5–8.2 runs ahead of the dye front and may represent an uncharacterized lipid whose synthesis (or fucosylation) is terminated near the end of meiosis. There are at least two round spermatid-specific components with molecular weights/isoelectric points of 45,000/4.7 and 80,000/7.2. When whole cell components were analyzed by two-dimensional electrophoresis, the results were virtually identical to those obtained with plasma membranes (Fig. 5).

DISCUSSION

This study has demonstrated that isolated spermatogenic cells of the adult mouse can be cultured for at least 24 h and retain viabilities in excess of 90% during this time. The conditions and medium used in this study are not optimal, however, for the long-term maintenance and the continued differentiation of these cells. Further improvement in these areas will require additional information concerning the nutritional and hormonal factors needed by mammalian germ cells. Undoubtedly, additional knowledge of the factors secreted by Sertoli cells will greatly benefit studies of spermatogenic cell differentiation.

Besides maintaining acceptable viabilities (>90%), isolated and cultured mouse spermatogenic cells are capable of synthesizing and fucosylating multiple

cellular polypeptides. Other groups have looked at the synthesis of proteins by rat spermatogenic cells utilizing either intratesticular injections of [^{35}S]-methionine (Kramer and Erickson, 1982) or 3-h incubations of isolated cells with [^{35}H]leucine (Boitani et al., 1980). Recently, Jutte et al. (1985) examined whole-cell protein synthesis by pachytene spermatocytes and round spermatids. Round spermatids were cultured for only 2 h, and cell viabilities were not indicated. Furthermore, in these studies, no subcellular fractionations were undertaken to determine what plasma membrane components were synthesized.

We have demonstrated here that proteins incorporated into the plasma membrane are qualitatively the same ones present in the plasma membranes prepared from freshly isolated cells. There are some quantitative changes in the synthesis of certain proteins, but actual measurement of these changes is

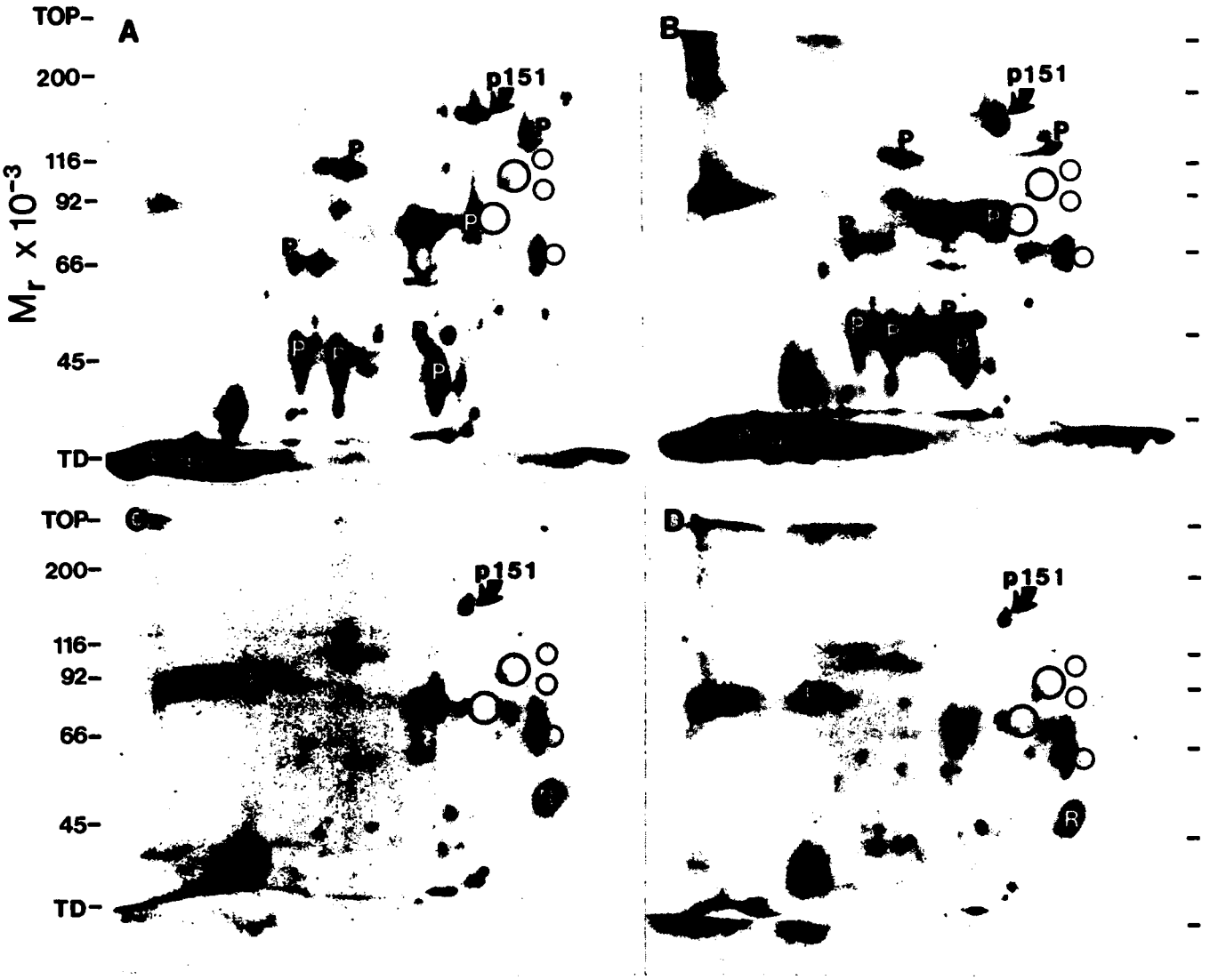

FIG. 5. Two-dimensional gel analysis of fucose incorporation into components of whole spermatogenic cells and plasma membranes. Populations of pachytene spermatocytes and round spermatids (each more than 90% pure) were cultured in 40–60 μCi/ml [6-^{3}H]-L-fucose. After 24 h, cells were harvested and washed with medium. One tenth of the cells were pelleted and saved for analysis of total cellular incorporation. Plasma membranes were prepared from the remainder of the cells. Samples were analyzed by two-dimensional gels (Minigels, Idea Scientific, Corvallis, OR) using a second dimension of 7% polyacrylamide, and stained and autofluorographed as described in the text. Only the autofluorographs are shown. (*A*) Whole pachytene spermatocytes; (*B*) plasma membranes from pachytene spermatocytes; (*C*) whole round spermatids; (*D*) plasma membranes from round spermatids. For reference, *large circles* indicate the positions of *p95* and *p75*, *small circles* indicate the positions of *c1*, *c2*, and *p55* (compare with *Fig. 3*). Constituents fucosylated only by isolated pachytene spermatocytes are labeled (*P*), while those fucosylated only by isolated round spermatids are labeled (*R*). *P-gl* represents a presumptive lipid synthesized or fucosylated only by pachytene spermatocytes. *TD* indicates the position of the tracking dye.

beyond the scope of this study. In addition, we do not know whether the isotopes used in this study have equilibrated to the same extent in amino acid and sugar pools of the spermatocytes and spermatids. Long-term labeling periods were used in this study, so we doubt whether different rates of equilibration between the two cell types would greatly affect the relative amounts of incorporation of isotope into proteins and glycoproteins.

However, one major quantitative change is worthy of further discussion. One of the major Coomassie-blue-staining and silver-staining proteins, p151, is present in both pachytene spermatocytes and round spermatid plasma membranes. Surprisingly, little or no p151 is synthesized by the latter cell type, suggesting that p151 synthesis is dramatically altered after meiosis. Whether this alteration is due to transcriptional or translational controls has yet to be determined, but a detailed study of the synthesis of this component may yield important information concerning the synthesis of proteins during spermatogenesis.

The function of p151 is not known at this time. However, we have noted (Millette and Scott, 1984) that p151 has a molecular weight and Concanavalin-A-binding properties similar to a major desmosomal glycoprotein (Shida et al., 1982; Cohen et al., 1983; Cowin and Garrod, 1983) and that its differential synthesis is what one might expect if p151 is involved in desmosomes between Sertoli cells and germ cells (Russell, 1980). As determined by cell-surface iodination, p151 is exposed on the external surface of pachytene spermatocytes and round spermatids (Millette and Moulding, 1981b) and potentially could be involved in interactions with other cells.

The fucosylation of a glycoprotein with a molecular weight and isoelectric point similar to p151 does not correlate with the synthesis of the polypeptide backbone to this component (Fig. 5). It is possible that p151 can be fucosylated long after it has been incorporated into the plasma membrane, perhaps by fucosyl transferases on the cell surface or in the culture medium. The fucosylation that we detect in round spermatids may also be due to the small amounts of p151 synthesized by these cultures. Alternatively, the fucosylated component that we detect at this position may be another glycoprotein with a molecular weight and isoelectric point very similar to those of p151. The resolution of these possibilities awaits the development of specific immunological probes for p151.

In light of previous papers describing the minor differences in plasma membranes from pachytene spermatocytes and round spermatids, the results showing very distinctive fucose labeling patterns for these two cell types were quite unexpected. These results imply that the differentiation of spermatogenic cells may be associated with the appearance or disappearance of specific glycoconjugates.

Our results showing cell-type-specific fucosylation by mouse pachytene spermatocytes and round spermatids do not totally agree with a similar study done with rat spermatogenic cells (Grootegoed et al., 1982). In this study, as in ours, more than 15 components were fucosylated by rat pachytene spermatocytes and round spermatids in culture for 20 h. Aside from the major band that migrated ahead of the dye front, no qualitative differences were detected by Grootegoed et al. (1982) in autofluorograms of one-dimensional gels generated with samples from pachytene spermatocytes and round spermatids. In our study, we may have detected cell-type-specific fucosylation for two reasons: 1) there may exist a species difference in the biosynthetic patterns of mouse and rat germ-cell-surface glycoproteins, and/or 2) we have analyzed our samples by high-resolution gel electrophoresis in two dimensions.

Previous investigators also concluded that the fucose was incorporated into acrosomal constituents and that "the synthesis of acrosomal glycoproteins is initiated during the prophase of meiosis in spermatocytes" (Grootegoed et al., 1982). As demonstrated by autoradiography at the level of the electron microscope, fucose does accumulate in glycoconjugates of the developing acrosomes of rat spermatids (Clermont and Tang, 1985). On the other hand, two possibilities are suggested by our demonstration that the patterns of fucose incorporation into male germ-cell plasma-membrane constituents are virtually identical to those from whole cells. The first is that, as found in studies with other cells (Yurchenko et al., 1978), fucose is incorporated primarily into plasma membrane glycoconjugates. The second possibility is that some acrosomal membranes co-purify with plasma membranes of these cells. In fact, a monoclonal antibody made against spermatogenic cell membranes prepared by the procedure used in this study reacts with an intracellular component of the developing acrosome of mouse spermatids (O'Brien et al., 1985). Actual assignment to the acrosome of any of the fucosylated

components detected in our study or that of Grootegoed et al. (1982) must await the development of specific immunological and genetic probes for the identification of acrosomal constituents.

In addition to glycoproteins, other components such as glycolipids and glycosaminoglycans could conceivably be fucosylated by mouse spermatogenic cells. It is very likely that the component that migrates ahead of the dye front in gels of pachytene spermatocyte samples is a glycolipid. A precedent for this possibility is a fucosylated ganglioside isolated from boar testes (Suzuki et al., 1975). Another lipid, sulfatoxygalactosylacylalkylglycerol, is synthesized during specific stages of spermatogenesis (Letts et al., 1978). This and other lipids from male germ cells are highly antigenic (Lingwood and Schachter, 1981; Teuscher et al., 1982; O'Brien and Millette, 1984), yet their roles in reproduction are not known. High-molecular-weight lactosaminoglycans exist on the surfaces of male mouse germ cells (Fenderson et al., 1984) but if they are fucosylated, they probably would not have been detected in our gels because of their size. Presumably these are the same lactosaminoglycans that are found associated with sperm and are subsequently fucosylated in vivo by an epididymal transferase (Cossu and Boitani, 1984).

At least two possibilities exist to explain the differential fucose labeling patterns seen with pachytene spermatocytes and round spermatids. Either there are different substrates available for the fucosyl transferases present or there exist transferases that are specific to one cell-type or another. A GDP-L-fucose: glycoprotein-fucosyl transferase activity has been found in mouse spermatogenic cells but its actual specificity has not been characterized (Letts et al., 1974). In the previous study, overall transferase activity was found to decline after the spermatocytes had completed meiosis to become spermatids. This decline in enzyme activity might be related to the decrease we see in the complexity of the fucosylation patterns of round spermatids compared to those of pachytene spermatocytes.

We have shown here that mouse spermatogenic cells can be cultured for at least 24 h and that during this time they can synthesize plasma membrane components, some of which are fucosylated in a cell-specific manner. Other studies on the formation of flagella by cultured round spermatids of the mouse (Gerton and Millette, 1984) and recent investigations with cells from the newt (Kiyotaka and Abe, 1983), Xenopus (Risley, 1983), and rat (Tres and Kierszenbaum, 1983) suggest that the analysis of limited periods of spermatogenesis in vitro are possible. Further developments on the culture of isolated male germ cells and the co-culture of germ cells with Sertoli cells (Tres and Kierszenbaum, 1983; D'Agostino et al., 1984; Hadley et al., 1985) will aid our understanding of the cell-surface events of spermatogenesis.

ACKNOWLEDGMENTS

We thank Mr. Steve Borack of the Photographic Unit and Ms. Kayte Taudel for assistance in preparing the manuscript, and Mr. B. Keyes Scott for technical assistance.

Animals used in this study were maintained in accordance with the guidelines of the Harvard Medical School Committee on Animals and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. 78-23 [National Institutes of Health], revised 1978).

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