Summary
Spermatogenesis, a complex process of cellular differentiation involving germ cell proliferation and renewal, meiosis and spermiogenesis, provides a unique system for the study of cell differentiation in mammals.

The most important event during spermatogenesis is the meiotic cell division which results in the formation of haploid spermatids from diploid spermatocytes. The reduction in the number of chromosomes to half is accompanied by several metabolic changes in the evolving germ cell and therefore germ cells at these two stages of spermatogenesis (spermatocytes and spermatids) has attracted the attention of several investigators.

While spermatogonia thrive on glucose, spermatocytes and spermatids experience a rapid drop in their ATP levels in glucose supplemented media and require lactate and/or pyruvate for maintaining normal energy levels. However spermatozoa regain the ability to utilize glucose for energy. The two germ cell types (spermatocytes and spermatids) not only show anomalous metabolic activity, but they also have a very special location within the seminiferous tubules. These two stages are placed in the adluminal component of the blood testis barrier and remain embedded in the Sertoli cell cytoplasm throughout their life, protected from the general circulation.

With a view to study the changes occurring in germ cell metabolism and macromolecular organisation of cytosolic and surface proteins in the meiotic and post-meiotic germ cells, the present study was designed to purify rat spermatocytes and spermatids and study them in vitro.

Though considerable amount of work has been done on germ cell differentiation during spermatogenesis using the whole testis tissue, work on isolated germ cell was initiated only during the past decade. In the present study we have attempted to isolate highly purified population of spermatocytes (meiotic germ cell) and spermatids (post-meiotic germ cell) by a two step separation technique. The study on isolated germ cell is important since it helps us in understanding the nature of factors that regulate the morphological and biochemical characteristics related to the germ cell proliferation, differentiation, metabolism and their survival.

In the first series of experiments, we adapted a method to obtain purified population of spermatocytes and spermatids from adult male rats. A sophisticated technique of 'Centrifugal Elutriation' was standardised to separate the cells by the difference in their sedimentation...
SUMMARY..... CONTD.

tation constants which was followed by density gradient centrifugation on Percoll. Decapsulated testis tissue was digested with trypsin to release the testicular cells in a suspension which was subjected to centrifugal elutriation to obtain ~ 75% pure sample pachytene spermatocytes and round spermatids in a single step. These fractions were further purified on linear Percoll gradients by centrifugation to obtain > 90% pure fractions of spermatocytes and spermatids. More than 97% of the cells prepared by this method excluded trypan blue showing viability and spermatids were found to be comparatively more resistant to cellular damage in vitro. This two step separation technique for germ cell was found to be superior to the method used previously i.e. unit gravity sedimentation (STAPUT). The cells separated by elutriation remain in isotonic cell culture medium and are not exposed to high and low tonicity of gradients during separation and therefore much higher yields of viable cells could be obtained in less time.

In the second series of experiments, key enzymes of carbohydrate metabolism viz., glycolysis, HMP pathway and TCA cycle were estimated in isolated and purified rat spermatocytes and round spermatids to investigate the changes that occur in the genetic expression of metabolic enzymes in the germ cell types during the process of meiosis. The study indicates that the enzymes of glycolytic pathway and pentose phosphate pathway exhibited significantly higher activities in spermatocytes as compared to spermatids. In contrast, the oxidative pathway (TCA cycle) showed lower activity in former than in the latter. Thus the germ cells undergoing meiosis experience a marked change in the type of energy metabolism and the equilibrium between anaerobic and aerobic metabolism shifts more in favour of aerobic metabolism in the post-meiotic germ cells. Our present findings support the earlier findings that normal levels of ATP are maintained in spermatids exclusively in the presence of lactate while spermatocytes can utilize both lactate and/or pyruvate, (while retaining significant ability to utilize glucose).

In the third series of experiment the activities of several key lipid metabolising enzymes in the purified fractions of adult rat spermatocytes and spermatids were estimated to establish the inherent potential of germ cells to undergo lipid remodelling during meiosis. The key anabolic enzymes (ATP citrate lyase and acetyl-CoA carboxylase) showed significantly higher activities in spermatids as compared to spermatocytes, while the catabolic enzymes (hydroxyacyl-CoA dehydrogenase, carnitine acetyl transferase) were more active in spermatocytes. It, therefore appears that meiosis results in an increase in fatty acid synthesis and decrease in fatty acid oxidation. While most of the lipid oxidizing enzymes showed significantly lower activity in post-miotic germ cells, ß-hydroxybutyrate dehydrogenase showed a visible increase in spermatids over spermatocytes. The higher activity of ß-hydroxybutyrate dehydro-
genase is usually encountered in tissues with very low carbohydrate metabolism and a high rate of fatty acid and/or ketone body oxidation. The low glycolytic potential of spermatids as compared to spermatocytes may compel the post-meiotic germ cells to oxidize fatty acids/ketone bodies as fuel molecules. The present study demonstrates that the germ cells undergo a major reorganisation in the fatty acid turnover during meiosis resulting in the shift towards fatty acid synthesis.

In the last series of experiments purified cytosolic and plasma membrane fractions were obtained from isolated spermatocytes and spermatids and their protein patterns were studied on SDS gels by electrophoresis.

The protein patterns of isolated spermatocytes and spermatids (whole cells) indicate that the total number of polypeptide bands in spermatids to be more as compared to spermatocytes, which contained a higher amount of protein per cell. A comparative study on the protein pattern of cytosolic fraction of the two cell types indicate the presence of two polypeptide bands of 16 KD and 21.5 KD exclusively in spermatocytes while a protein band of 14.0 KD was seen only in spermatids. The study suggests changes in the genetic expression of cytosolic proteins during spermatogenesis resulting in the appearance of proteins specific to the haploid germ cells.

The study of protein patterns of plasma mambranes from purified populations of spermatogenic cells allowed a direct biochemical analysis of cell surface markers. Spermatocytes showed the presence of some prominent protein bands (61.5 KD, 55 KD, 52 KD and 45 KD) which were absent or very faint in spermatids. Similarly spermatids contained a few protein bands of 100 KD, 70 KD, 48.5 KD, 35 KD and 24 KD which were absent in spermatocytes. These alterations in membrane proteins may be responsible for the alterations in the attachment factors of germ cells required for adherence to the Sertoli cells and paracrine communication between the two cell types.

THE HIGHLIGHTS OF THE PRESENT STUDY ARE:-

(1) The purification of germ cell types to near homogeneity by elutriation and density gradient centrifugation provides useful tool for studying specific germ cell types and their biochemical characterization.

(2) Spermatids prefer aerobic pathway of energy metabolism over glycolysis (as is seen by higher activities of TCA cycle enzymes). This is possibly the reason for their preferential utilization of lactate over glucose.
(3) Spermatocytes exhibiting higher activities of glycolytic enzymes as compared to spermatids supports the earlier view that the spermatocytes not only utilize lactate to the same extent as pyruvate, but also retain significant ability to oxidize glucose.

(4) The higher activity of glucose-6-phosphate dehydrogenase (HMP-Pathway) in spermatocytes may suggest the importance of this pathway to meet the needs of biosynthesis of nucleotides for RNA synthesis (by generating important products like NADPH and ribose 5-phosphate). Earlier studies have shown that active DNA synthesis occurs during primary spermatocyte stage when it acquires a tetraploid amount of DNA. Along with this there is also evidence of increased RNA and protein synthesis during this stage. Our study also suggests a drop in the biosynthetic potential of germ cells during meiosis.

(5) The study of lipid metabolism suggests that during meiosis the lipid turnover shifts in favour of fatty acid synthesis for providing spermatid specific lipids.

(6) The complete absence of the long chain acyl-CoA synthetase may make the germ cell types studied incapable of oxidizing free fatty acids, but the presence of β-hydroxyacyl CoA-dehydrogenase suggests that this enzyme may oxidize fatty acid which enter the cells as acyl-CoA esters. These acyl esters may enter the cells from the neighbouring Sertoli cells via the 'lipid cycle'.

(7) The higher activity of β-hydroxybutyrate dehydrogenase in spermatids suggests the cells tend to metabolize carbohydrate at a low level and prefer a higher rate of fatty acid and ketone body oxidation.

(8) The changes in protein patterns of cytosolic fraction of germ cells indicate changes in expression of cytosolic protein components during meiosis.

(9) The electrophoretic protein patterns of purified plasma membranes reveal differences in surface composition of the two germ cells which may be important in bringing about the changes in the attachment factors responsible for their adherence to Sertoli cells.

The present study clearly demonstrates that the germ cells undergoing meiosis experience a major reorganisation of their functional and structural parameters. While carbohydrate metabolism shifts in favour of oxidation, lipid metabolism shifts towards biosynthesis of sperm specific molecules (e.g., pentaenoic acid). Cytosolic and membrane protein pattern have indicated certain specific polypeptides expressed in spermatocytes and spermatids. A further study to characterize some of these proteins may lead to the identification of specific markers for these two important stages of spermatogenesis. It would be important to study the involvement of surface proteins in germ cell-germ cell and germ cell-Sertoli cell attachments and paracrine communications between the different cell types mentioned.
Bibliography
BIBLIOGRAPHY

Abou-Issa, H. and Reichert, L.E. (1976)
J. Biol. Chem. 251:3326

J. Reprod. Fertil. 54:67

Biochim. Biophys. Acta. 530:367

Lipids 14:262

Lipids 15:389

J. Histochem. Cytochem. 25:480

J. Cell Biol. 74:68

Recent. Prog. Horm. Res. 40:531


Bishop, D.W. (1967)
J. Gen. Physiol. 50:2504

Bishop, D.W. (1968)
J. Reprod. Fertil. 22:69

Blackshaw, A.W. (1970)


Boone, C.W., Harell, G.S and Bond, H.E. (1968)
J. Cell Biol. 36:369

Bosca, L., Aragon, J.J. and Sols, A. (1985)
J. Biol. Chem. 260:2100

Membrane Biochem. 2:17

Nature 234:301

Brooks, D.E. and Mann, T. (1972)
Biochem. J. 129:1023

Biochem. J. 156:527

Biochem. J. 174:741

Biol. Repord. 34:195

Burton, K. (1956)
Biochem. J. 62:315
Mol. Reprod. Dev. 43:403

Christensen, A.K. and Manson, N.R. (1965)  
Endocrinology. 76:646

Clausen, J. (1969)  
Biochem. J. 111:207

Clermont, Y. (1972)  
Physiol. Rev. 52:198


Electrophoresis 16(7):1225

Crabtree, B. and Newsholme, E. A. (1972a)  
Biochem. J. 126:49

Crabtree, B. and Newsholme, E.A. (1972b)  
Biochem. J. 130:697

Davis, J.C. and Schuetz, A.W. (1975)  
Exp. Cell. Res. 91:76

Davis, J.R., Morris, R.N. and Hollinger, M.A. (1964)  
Am. J. Physiol. 207:50

Davis, J.R. and Firlit, C.F. (1965)  
Annu. J. Physiol. 209:425

Biochem. J. 98:342

J. Reprod. Fertil. 14:407

Chromosoma (Berl) 83:409

Andrologia 15:236

Biol. Reprod. 3:308

Am. J. Anat. 128:265

Evan, R.W. and Setchell, B.P. (1978a)
J. Reprod. Fertil. 52:15

Evan, R.W. and Setchell, B.P. (1978b)
J. Reprod. Fertil. 53:357

J. Reprod Fertil. (Suppl) 10:105


Fiske, H. and SubbaRow, Y. (1925)
J. Biol. Chem. 66:375

Free, M.J. (1970)
Biol. Reprod. 14:481

Fritz, I.B. (1973)

Geremia, R., Boltani, C., Conti, M and Monesi, V. (1977)
Cell Differ. 5:343

Biol. Reprod. 35:1025

Glock, G.E. and Mclean, P. (1953)
Biochem. J. 55:400

Canad. J. Biochem. 49:753

Goldberg, E. (1977)

Grabske, R.J., Lake, S., Gledhill, B.L. and Meistrich, M.L. (1975)
J. Cell Physiol. 86:177

Lipids 17:604

Lipids 18:275

Biochem. J. 168:23

Gamete Res. 5:303

Biochem. J. 226:889

J. Reprod. Fertil. 77:99

J. Reprod. Fertil. 77:109


Annal. NY. Acad Sci. 564:232

Grootegoed, J.A. and Den Boer, P.J. (1990)

Ind. J. Exp. Biol. 31:305

Endocrine Res. 20:275

Int. Rev. Cytol. 62:187

Dev. Biol. 57:375

BIBLIOGRAPHY.... CONTD.

Hubscher, G. and West, G.R. (1968)
Nature. 205:799

Igarashi, M. and Hollander, V.P. (1968)
J. Biol. Chem. 243:6084

Nature. 192:1053

J. Reprod. Fertil. 62:399

J. Reprod. Fertil. 65:431

J. Reprod. Fertil. 73:497

Cytogenet. Cell Genet. 84:78

J. Reprod. Fertil. 64:139

Lacy, D. (1962)

Lacy, D. (1967)
Endeavour 26:101


Nature 227:680

(xii)
Proc. Natl. Acad. Sci. 5:192

Lehninger, A.L. (1978) 
In: Biochemistry, Kalyani Publishers, Ludhiana.

Lima-de-Faria, German, J., Ghatnekar, M., McGovern, J. and Anderson, L. (1968) 
Hereditas 60:249

Can. J. Biochem. 50:963

Loir, M. and Wyrobek, A. (1972) 
Exp. Cell Res. 75:261

Exp. Cell Res. 83:319

Exp. Cell Res. 92:499


J. Biol. Chem. 193:265

Endocrinology 49:8

Mann, T. (1964) 

Makler, A. (1978)  
Fertil. Steril. 30:313

J. Biol. Chem. 240:2197

Tissue and Cell 11:741

Meistrich, M.L. (1972)  
J. Cell Physiol. 80:299

Meistrich, M.L. and Eng, V.W.S. (1972)  
Exp. Cell Res. 70:237

Exp. Cell Res. 79:213

Exp. Cell Res. 92:231

Meistrich, M.L. (1977)  

Biol. Reprod. 25:1065

Arch. Biochem. Biophys. 176:250

J. Cell Physiol. 73:191

Millette, C.F. and Belivee, A.R. (1977)  
J. Cell Biol. 74:86
Millette, C.F. (1979a)

Millette, C.F. (1979b)
Current Top. Dev. Biol. 13:1

Dev. Biol. 79:309

J. Cell Sci. 43:279

Millette, C.F and Moulding, C.T (1981a)
Gamete Res. 4:317

J. Cell Sci. 48:367

Endocrinology 110:1535

Biol. Reprod. 26:445

Mollenhouer, H.H. (1964)
Stain Technology 39:111

Monesi, V. (1962)
J. Cell Biol. 14:1

Monesi, V. (1965)
Exp. Cell Res. 39:197

Monesi, V. (1971)
J. Reprod. Fertil. (Suppl) 13:1
Montamat, E.E. and Blanco, A. (1976)
Exp. Cell Res. 103:241


Munro, H.N. and Fleck, A. (1966)
Meth. Biochem. Anal. 14:113

Biochim. Biophys. Acta 447:474

J. Cell Biol. 79:1

J. Biol. Chem. 255:2907

J. Biochem. 90:933

J. Biol. Chem. 257:13945

Am. J. Physiol. 247:234

Biol. Reprod. 30:1187

Andrologia 16:446

Andrologia 17:461
BIBLIOGRAPHY... CONTD.

Dev. Gr. Differ. 28:489

Biol. Reprod. 35:927

Andrologia 19:91

Nyquist, S.E., Acaff, K. and Mollenhauer, H.H. (1975)
Biol. Reprod. 8:119

Dev. Biol. 101:307


O'Rand, M.G. and Romrell, L.J. (1977)
Dev. Biol. 55:347

O'Rand M.G. and Romrell, L.J. (1980)
Dev. Biol. 75:431

Endocrinology 112:115

Lab Med. 15:740

Paul, H.E., Paul, M.F., Kopko, F., Bender, R.C. and Everett, G. (1953)
Endocrinology 53:585

(xvi)

Anal. Biochem. 88:271

Fertil. Steril. 21:151

Peterson, R.N. and Freund, M. (1976)  

J. Biol. Chem. 250:5791

J. Biol. Chem. 256:7150

Racker, E. (1950)  
Biochem. Biophys. Acta 4:211

Rabinowitz, R. and DeBernard, B. (1957)  

Radu, I. and Voisin, G.A. (1975)  
Differentiation 3:107

Read, G., Crabtree, B. and Smith, G.H. (1977)  
Biochem. J. 164:349

Reynolds, E.S. (1963)  
J. Cell. Biol. 17:208

Biol. Reprod. 24:1032
Dev. Biol. 49:119

Romrell, L.J. and O’Rand, M.G. (1978)  
Dev. Biol. 63:76

Gamete Res. 3:179


Setchell, B.P. (1970)  
J. Reprod. Fertil. 23:79

J. Reprod. Fertil. 48:301

Singh, E.L. and Hare, W.C.D. (1980)  
Mamm. Chrom. 21:120

Nature (London) 184:249


Steinberger, A., Steinberger, E. and Perloff, W.H. (1964a)  
Exp. Cell Res. 36:19

Endocrinology 74:788

Steinberger, A. and Steinberger, E. (1966a)  
Exp. Cell Res. 44:443
Steinberger, A. and Steinberger, E. (1966b)
Exp. Cell. Res. 44:429

Steinberger, A. and Steinberger, E. (1967)
J. Reprod. Fertil. (Suppl) 2:117


p. 333.

In: Methods in Mammalian Embryology. Ed. J. C. Daniel. Sanfrancisco: W.H Freeman,
p. 496.

Biol. Reprod. 28:483


Tanaka, T., Hosaka, K. and Numa, S. (1979)

Proc. Natl. Acad. Sci. 80:3377

Dev. Biol. 64:297

Tung, K.S.K., Han, L.P.B. and Evan, A.P. (1979)
Dev. Biol. 68:224


J. Biol. Chem. 252:1303

Can. J. Bioch. 49:761

Waites, G.M.H. and Setchell, B.P (1969) 
Adv. Reprod. Physiol. 4:1


Watson, M.L. (1958) 
J. Biophys. Biochem. Cytol. 4:474

Endocrinology 91:415

Yamazaki, M. and Hayaishi, O. (1968) 
J. Biol. Chem. 243:2934

Zammit, VA. and Newsholme, E.A. (1976) 
Biochem. J. 160:447