MAGNETIC RESONANCE IN MEDICINE 23, 264–274 (1992)

The Progression of Spermatogenesis in the Developing Rat Testis followed by ^{31}P MR Spectroscopy

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Received May 15, 1990; revised December 28, 1990

To evaluate the use of human testicular ^{31}P MR spectroscopy as a diagnostic tool to differentiate between several stages of male infertility, we have studied the testicular levels of several phosphorus containing compounds in the rat in relation to the condition of spermatogenesis and the cell types present in the seminiferous tubules of the testis. During testicular maturation several characteristic changes occur in the ^{31}P MR spectrum of the testis of male Wistar rats. The phosphomonoester/adenosine triphosphate (PM/ATP) ratio shows a decline from 1.61 to 1.02 between the age of 3 and 12 weeks, whereas the phosphodiester (PD)/ATP ratio increases from 0 to 0.72. The testicular pH increases in the same time from 7.06 to 7.32. Testicular MR data obtained after 12 weeks of age onward do not show significant change anymore. The high PM/ATP ratio is associated by a relative high amount of proliferating spermatogonia and spermatocytes during meiosis in the testis, whereas the PD peak seems to be correlated with the release and maintenance of spermatozoa. The MR spectra show a specific fingerprint in all developmental stages of the rat testis as a result of the different cell types in the testis.

INTRODUCTION

Assessment of impairment of spermatogenesis, in case of idiopathic oligozoospermia or azoospermia, must often rely on an invasive surgical approach, i.e., biopsies (*1*). To avoid unnecessary surgical exposure of the testis which could possibly cause extra damage to spermatogenesis (*2, 3*), an accurate, nondetrimental method to assess the testicular function needs to be developed.

In vivo MR spectroscopy has proved to be a powerful tool to obtain, noninvasively, metabolic information from organs like brain, heart, liver, kidney, and skeletal muscle (*4–11*). MR spectroscopy could also be an important approach to study testis function, but little is known about the relation between testicular MR spectra and the condition of spermatogenesis. Preliminary data of a nondisturbed human (*12–14*), rat (*15*), and dog spermatogenesis (*16*) show a high intensity of the phosphomonoester peak (PM), whereas its intensity decreases in case of spermatogenic suppression (*12–16*).

In general, the PM peak represents phosphocholine and phosphoethanolamine, AMP, and sugar-phosphates (*17–19*). The testicular level of PM, in the adult rat, was

0740-3194/92 $3.00

found to consist mainly of phosphocholine and phosphoethanolamine (*20*). Presumably, the concentration of these phospholipid precursors might depend on the relative amount of cell proliferation or differentiation (*18, 21*) and in the testis on the condition of spermatogenesis. The testicular levels of phosphodiesters (PD), which are considered to be catabolic products of phospholipids (*17–19*), might also depend on the condition of spermatogenesis.

To understand changes of the PM/adenosine triphosphate (ATP) and PD/ATP ratio in the testis and to determine the individual contribution of the interstitial cells and the different cells inside the seminiferous tubules to the ^{31}P MR *in vivo* spectrum, a detailed study is necessary. The maturing rat testis seems to offer a useful system, because the effect on the MR spectrum of the appearance of new cell types in the testis can easily be studied. Hence, we compared the morphology of the testis during different stages of rat testis development with the corresponding *in vivo* ^{31}P MR spectra. Furthermore, the ability to manipulate the cellular composition of the testis in the rat model, by different treatments, could facilitate the basic understanding of the changes in the ^{31}P MR spectrum of the testis. To determine the composition of the PM and PD resonances, perchloric acid (PCA) testicular extracts were prepared at the age of 3, 6, and 12 weeks.

MATERIALS AND METHODS

Animal Model

Fourteen age groups, each consisting of 5 male Wistar rats, at the ages of 3, 6, 7.5, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, and 21 weeks, were used for MR spectroscopic examination. Testes of rats younger than 3 weeks could not be investigated because of their small size. The animals were weighed and anesthetized with 5 mg/kg stesolid (Dumex) intraperitoneally and 0.5 ml/kg hypnorm (Janssen pharmaceutica) intramuscularly. To avoid retraction of the testis into the inguinal canal or the lower abdomen during the MR spectroscopy experiment, testes of the anesthetized rats were fixed using a piece of tape applied around the neck of the scrotum. The time limit of the experiment was about 45 min, because it was almost impossible to keep rats of 3 and 6 weeks under anesthesia for longer than 45 min. Longer anesthesia times resulted in high mortality. After the MR spectroscopy experiment the animals were killed and the testes were removed, weighed, and fixed in Bouin's solution for histological analysis. To investigate the effects of the anesthesia on the pattern of the MR spectra, a group of 6 adult male Wistar rats was investigated weekly during 6 weeks. The obtained MR spectra of these rats were compared with corresponding MR spectra of rats of the same age, but which underwent only one examination.

MR Methods

^{31}P MR spectra were obtained on a Bruker MSL 200 spectrometer equipped with a 4.7-T wide bore (150 mm) vertical magnet operating at a frequency of 81.015 MHz. A single-tuned ^{31}P single turn surface coil with a diameter of 9 mm (used in experiments with rats at the age of 3 weeks) and a 15-mm coil (other ages) was placed directly on the scrotum and was used for excitation and data acquisition. Shimming of the magnetic field was performed on the H_2O proton signal with the same coil. The ^{31}P spectra

were obtained from 256 accumulated free induction decays after pulses of 12 μs. Longer pulse times resulted in an increasing muscle contamination originating from the rats tail. The data were accumulated using 2K data points, 5000 Hz spectral width, and a repetition time of 2 s. The spectra were analyzed after deconvolution and exponential multiplication resulting in 18 Hz line broadening. Quantitition of the metabolites was achieved by integrating peaks of interest. The concentration of the metabolites was expressed relative to the ATP concentration. The β-phosphate peak of ATP was used to determine ATP levels. Peak ratios were not corrected for partial saturation effects. Considering the length of these experiments, which exceeds 3 h, the duration of the time of anesthesia is much too short to perform fully relaxed measurements.

The calculation of the intracellular pH was based on the chemical-shift dependence of inorganic phosphate (P_i) and phosphocreatine (PCr) (*22*). The chemical shift of the PCr resonance was assigned to 0 ppm.

To check the volume sampled by the generated pulse in the spectroscopy experiment, we clamped the spermatic cord after some experiments, without changing the position of the animal, in order to cause ischemia of the testis. ATP signals originating from the testicle would disappear, whereas ATP signals originating from the scrotum or skeletal muscle would still show up.

Testicular PCA extracts were prepared (*20*) of rats at the age of 3, 6, and 12 weeks. Fully relaxed ^{31}P MR spectra were recorded in a 10-mm NMR tube with a repetition time of 20 s on the same system.

The phosphate compounds were identified by adding known quantities of pure compounds, by adjustments of the pH observing the chemical-shift pH dependence of the separate compounds, and by comparison with spectra of PCA extracts of other tissues (*20*).

Histological Procedures

Testes were fixed in Bouin's fluid. After dehydration, the material was embedded in Technovit 7100 plastic (Kulzer and Co, GmbH, Wehrhein, FRG), a glycol methacrylate. Five-micrometer sections were cut at least 1 mm away from the edge where the testis had been cut in half, in order to avoid distorted tubular compartments. The sections were stained by the periodic acid-Schiff (PAS) technique and Gill's hematoxylin (Polysciences Inc., Warrington, PA). The diameter of the testicular tubules was determined by means of a light microscopic optic lath. For each rat the tubular diameter was obtained by determination of 10 diameters of tubular cross sections in each of five sections.

Statistical Analysis

Results of the MR spectroscopy experiments and the measurements of the tubular diameter are presented as means $\pm$ SD. Statistical analysis was performed using the unpaired Student's t test.

RESULTS

Histology

Histological sections of rats at the age of 3, 6, 9, and 12 weeks are shown in Fig. 1. In the seminiferous tubules of rats at the age of 3 weeks, type A, intermediate, and

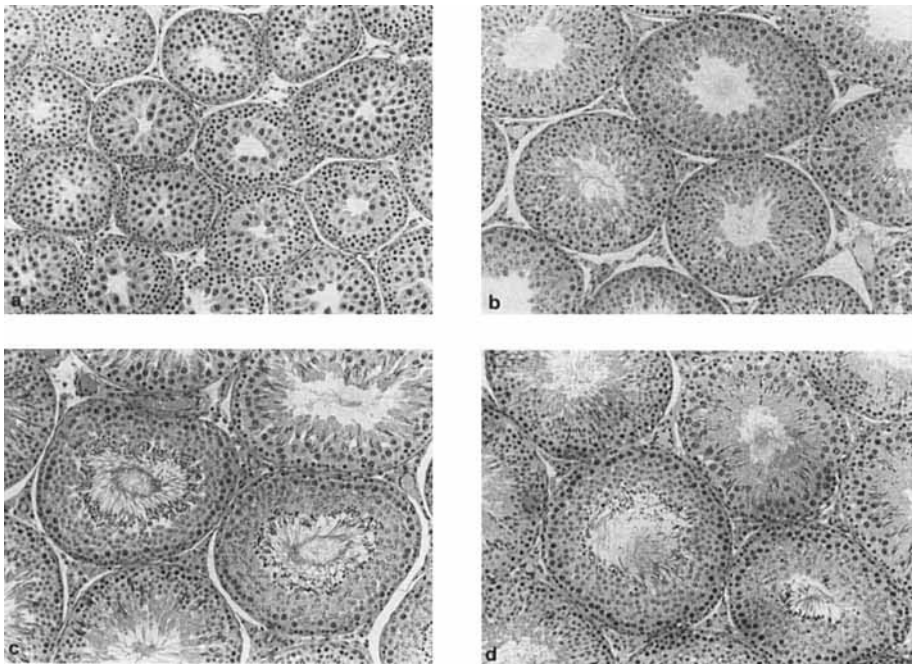

FIG. 1. Histological sections of the testis of the rat at the age of 3 (a), 6 (b), 9 (c), and 12 weeks (d).

type B spermatogonia as well as preleptotene, leptotene, zygotene, and pachytene spermatocytes could be observed. In some tubuli round spermatids could be seen, occurring in early stages of the cycle of the seminiferous epithelium. The histological sections of the testis of the rat at 6 weeks showed, in addition to the cell types which were observed in the testis at the age of 3 weeks, round and elongated spermatids. Very little or no release of spermatozoa was observed at this age. At the age of 9 and 12 weeks spermatogenesis is fully developed. In the corresponding histological sections

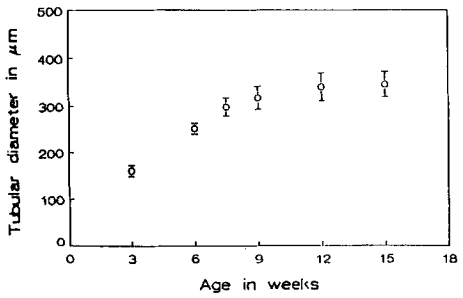

FIG. 2. Tubular diameter of the testis of the rat during testicular maturation. Values are the mean ± SD of 50 measurements.

of the testis, the release of spermatozoa can easily be seen as well as the occurrence of residual bodies. These residual bodies can be recognized as small dark dots and are located near the lumen.

The diameters of the testicular tubules of the rats at the age of 3, 6, 7.5, 9, 12, and 15 weeks are shown in Fig. 2. The tubular diameter was found to increase up to about 9 weeks after birth and was estimated to be 171 ± 12, 241 ± 19, 288 ± 19 to 327 ± 24 μm, at 3, 6, 7.5, and 9 weeks of age, respectively. There was no further significant increase in tubular diameter at later ages. The testicular weight and the body weight of the rats increased rapidly until the age of 12 weeks and much slower after 12 weeks. The data on the body and testicular weights are summarized in Table 1.

^{31}P MR Data

The ^{31}P MR spectrum of the rat testis contains PM, P_i, PD, PCr, and three ATP resonances, as indicated in Fig. 3. These resonances are characteristic for the testis except for PCr, which shows up in most of the ^{31}P MR spectra. PCr is not a component of the testis (*20*), but is observed *in vivo* because of muscle contamination, caused by the m. cremaster or by the muscles of the rat's tail.

A marked decrease of the PM peak and an increase of the PD peak during testicular maturation were observed. The decrease of the PM/ATP ratio occurs between the age of 3 and 10 weeks (Fig. 4). After 10 weeks no further changes in this ratio were found ($P < 0.05$).

The testicular PD/ATP ratio shows a considerable increase between the age of 6 and 12 weeks (Fig. 5). From the age of 12 weeks onward, the PD/ATP ratios do not show significant differences ($P < 0.05$) with older rats. The area of the PD peak of rats of 3 weeks could not be determined because of the relatively low signal to noise ratio at this age.

TABLE 1

Body and Testicular Weight of the Male Wistar Rat during Growth

Age	Body weight (g)	Testis weight (g)
3 weeks	48 ± 4	0.38 ± 0.06
6 weeks	150 ± 6	1.88 ± 0.07
7.5 weeks	195 ± 7	2.22 ± 0.10
9 weeks	245 ± 14	2.44 ± 0.12
10 weeks	268 ± 12	2.56 ± 0.14
12 weeks	276 ± 16	2.79 ± 0.10
13 weeks	286 ± 12	2.85 ± 0.12
14 weeks	294 ± 9	2.96 ± 0.14
15 weeks	299 ± 14	3.01 ± 0.11
16 weeks	301 ± 12	3.05 ± 0.15
17 weeks	320 ± 12	3.12 ± 0.12
18 weeks	322 ± 15	3.20 ± 0.16
19 weeks	329 ± 13	3.27 ± 0.15
21 weeks	335 ± 23	3.33 ± 0.21

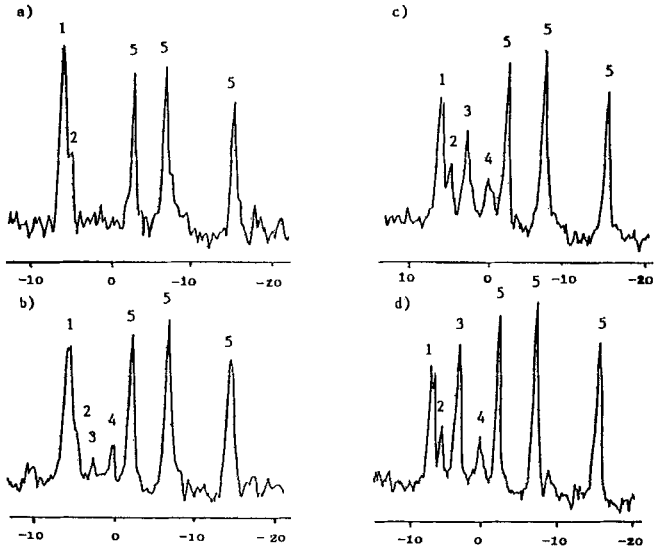

FIG. 3. ^{31}P MR *in vivo* spectra at 4.7 T (256 averages and 2 s repetition time) of the testis of the rat at the age of 3 (a), 6 (b), 9 (c), and 12 weeks (d). Peaks are assigned to: phosphomonoesters (1), inorganic phosphate (2), phosphodiesters (3), phosphocreatine (4), and γ, α, and β ATP (5).

The testicular P_i/ATP ratio did not show any significant variation during testicular maturation and amounted to 0.22 ± 0.07. Figure 6 shows the effect of testicular maturation on the intracellular pH of the testis. The testicular pH increases from 7.08 ± 0.10 at the age of 3 weeks to 7.32 ± 0.06 for 12-week-old rats. The testicular pH of rats from the age of 12 weeks onward does not show statistically significant ($P < 0.05$) differences as compared with older rats. Comparing single with repeated anesthesia, no significant differences in the MR data of the examined animals were found.

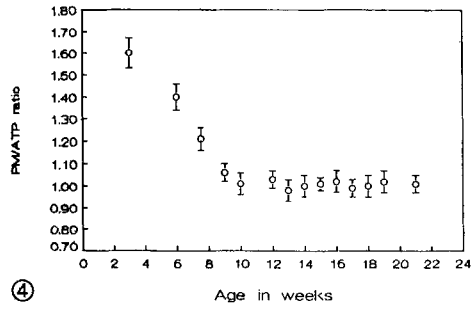

FIG. 4. The PM/ATP ratio of the testis of the rat during testicular maturation. Values are the mean ± SD of five experiments.

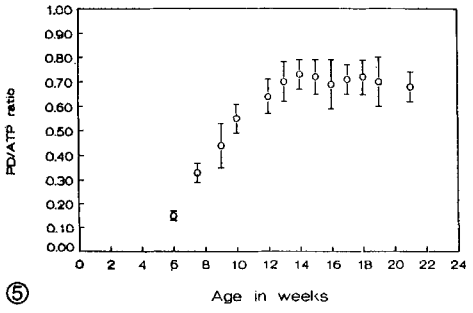

FIG. 5. PD/ATP ratio of the testis of the rat during testicular maturation. Values are the mean ± SD of five experiments.

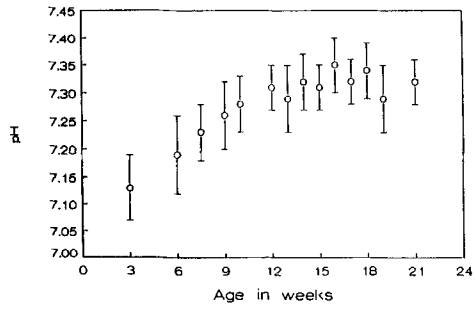

FIG. 6. The pH of the testis of the rat during testicular maturation. Values are the mean ± SD of five experiments.

The testicular ^{31}P MR spectrum of the rat in which the spermatic cord was clamped during the MR experiment showed only the PM peak and a very intense P_i peak. No ATP or PD resonances could be detected. The absence of ATP in these MR spectra shows the little contamination by surrounding tissue.

Figure 7 shows the low field portions of the PCA extract spectra of rat testes of rats at the age of 3, 6, and 12 weeks. In the testis of 3- and 6-week-old rats no GPC (peak D) or GPE (peak E) could be seen. Whereas, in the spectrum of the 12-week-old rat, a large amount of GPC and a small amount of GPE can be seen. The PM resonances were assigned only to phosphoethanolamine (peak A) and phosphocholine (peak B). No other components could be assigned in the PM region. Peak C was assigned to inorganic phosphate.

DISCUSSION

The PM/ATP Ratio

PCA extract analysis of the testis at the age of 3 weeks demonstrates that the PM peak originated from phosphocholine and phosphoethanolamine. These compounds

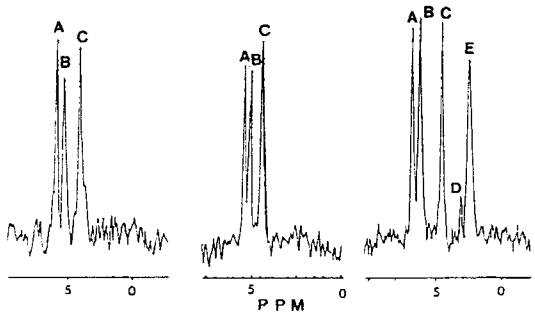

FIG. 7. ^{31}P NMR spectra at 4.7 T, proton broad-band decoupled, 64 averages and 20 s repetition time, of perchloric acid extracts of the testes of a rat at the age of 3, 6, and 12 weeks, on the left, middle, and right, respectively. Peaks are assigned to phosphoethanolamine (A), phosphocholine (B), inorganic phosphate (C), GPE (D), and GPC (E).

could also be detected in the testis of 6- and 12-week-old rats. No AMP or glycolytic intermediates could be assigned at all three ages.

Phosphocholine and phosphoethanolamine are precursors in biosynthesis of phosphatidylcholine and phosphatidylethanolamine, respectively, following the *de novo* Kennedy pathway (*23*). In rapid developing tissues, e.g., proliferating cells, the concentration of these phospholipid precursors might be elevated, caused by an increase of choline kinase activity (*18, 23, 24*), which finally results in an increased phospholipid biosynthesis. Bearing in mind the work of Kent and collaborators (*26–30*) and Vance and Pelech (*31*), which shows strong evidence that the synthesis of CDP-choline via the cytidyltransferase reaction is an important site of the regulation of phosphatidylcholine biosynthesis, the increase of the PMs could directly be correlated to the rate of phospholipid biosynthesis.

It is expected that in a proliferating cells system during meiosis, e.g., the rat testis at the age of 3 weeks, phospholipid biosynthesis is higher than in a cell system which also contains differentiating cells, e.g., the rat testis at the age of 6 weeks onward. Since the rate of phospholipid biosynthesis can be estimated from the increase in testis weight or tubular diameter, the changes in the PM/ATP ratio do not seem peculiar. Our preliminary studies indicate that the direct correlation between the PM intensity and phospholipid biosynthesis indeed exits in the rat testis. Since no glycolytic intermediates could be assigned in the PM peak, glycolytic changes could not be demonstrated when determining the intensity of the PM peak.

The Testicular pH

Although the standard deviation is large for each age, an increase in the intracellular pH in the testis during testicular maturation was observed. To our knowledge, this rise of intracellular testicular pH during maturation of the testis has not been reported before. If the rate of phospholipid biosynthesis increases, a high level of high energetic phosphates is demanded, following the *de novo* Kennedy pathway. Since it is known that glycolysis is the main energy supply of the testis (*32*), the rate of glycolysis and thus the $[H^+]$ production might also be elevated in a rapid growing testis.

The PD/ATP Ratio

The *in vivo* and the PCA extract experiments show that the PD peak is only present from the age of 6 weeks onward. At this point, histologically, the formation of elongated spermatids is complete and the transformation to spermatozoa commences. PCA extracts of the testis of the rat have shown that the PD peak mainly consists of GPC and, in much lesser amount, of GPE. Both are hydrolytic products of the major membrane phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), respectively. Since PC and PE are present in almost equal amounts in rat testicular tissue (*33*), equal amounts of GPC and GPE are also expected due to phospholipase A_1, A_2, and B and lysophospholipase activity. The great difference in GPC and GPE concentration might be caused by (age-dependant) differences in the activity of GPC-diesterase and GPE-diesterase (*34*). However, it might be questioned if the germ cells are responsible for this effect. Sertoli cells, in general, regulate spermatogenesis by controlling the biochemical environment in which the germ cells develop. Since it has

been shown that GPC is absent in active or mature cells and present in millimolar concentrations in resting and precursor cells (*35*), the high GPC concentration could also well be caused by the Sertoli cells. The rapid decrease of GPC in the ischemic testis also questions the assumption that the PDs act solely as pool for membrane phospholipid catabolites; in that case, at least a plateau level of phospholipid catabolites is expected during testicular ischemia.

Burt *et al.* have proposed a role for GPC in the control of membrane fluidity, via the negative feedback of GPC on lysophospholipase (*36*). Lysophospholipase is responsible for the formation of GPC out of lysophosphatidylcholine. The latter can increase membrane fluidity, which might be important for the release of spermatozoa into the seminal fluid and their transport through seminiferous tubuli. High GPC levels may also serve as an endogenous lysophospholipase inhibitor to preserve the phospholipid composition (*36*) of spermatozoa by increasing the flux of lysophospholipids that pass through the transacylase shunt (*37*). It has been shown that GPC is present in very high concentrations in seminal fluid (*38, 39*). Hinton and Setchell (*39*) also showed that the head of the rat epididymis possesses great GPC synthetic activity. Since the epididymis is used as transport as well as storage medium for spermatozoa, GPC seems to be essential in the medium for spermatozoa. Our results, which show a high GPC concentration in the testis, link well to the findings in these studies, in which a high GPC concentration in the seminal fluid was found. Other studies showed the importance of GPC in seminal fluid. A positive correlation was found between the GPC concentration and sperm motility (*40, 41*), whereas reduced GPC levels were found in semen of patients who underwent vasectomies (*42*). These considerations strongly suggest that the testicular PD peak mainly consists of extracellular GPC and that extracellular GPC is correlated with the formation and preservation of spermatozoa and in general with male fertility. Furthermore, overall germ cell degeneration has shown to be higher in a full grown testis than in a developing testis (*43*), which may also contribute to an increased PD/ATP ratio in older rats.

The testicular parameters, obtained using *in vivo* MR spectroscopy, are better parameters for age-dependent changes that the phospholipid content per gram testis weight. It has been shown that qualitative and quantitative analysis of the phospholipids of the testes does not give much information about the metabolic state of the testes. In the maturing rat testis, the phospholipid content relative to testis weight does not change significantly (*44–46*). Furthermore, in experiments in which spermatogenesis was suppressed using vitamin A deficient rats (*47*) or hypophysectomized rats (*48, 49*), the phospholipid content per testis weight did not show any significant difference with the control groups. It remains to be studied whether the testicular PM/ATP and PD/ATP ratios and pH are sensitive to suppression of spermatogenesis.

Conclusions

It can be concluded that ^{31}P MR spectroscopy can differentiate between several stages of spermatogenetic development in rats. A high PM peak is correlated to presence of a relative high number of proliferating spermatogonia and spermatocytes during meiosis. The appearance of more generations differentiating spermatogenic cells, such as round and elongated spermatids, lowers the PM peak. The PD peak seems to cor-

relate mainly with GPC in the seminal fluid. However, since the intensities of the PM and PD peak are dependent on the cell types inside the testis, clinical MR spectroscopy might discriminate between a normal functioning spermatogenesis and maturation arrest at different levels, germ cell aplasia, hypospermatogenesis, or obstructions in the reproductive tract. This discrimination is important in the diagnosis of testicular function, especially when male infertility is accompanied with serum hormone levels which are within normal limits. So far this discrimination can only be made with a testicular biopsy (*50*). MR spectroscopy might be a noninvasive alternative.

ACKNOWLEDGMENTS

The authors are grateful for the skillful technical assistance of Mr. P. van der Meer. We also thank Mr. H. van Dijk for providing experimental animals.

REFERENCES

1. D. L. GORDON, A. B. BARR, J. E. HERRIGEL, AND C. A. PAULSEN, *Fertil. Steril.* **16,** 552 (1965).
2. S. GUAZZIERI, A. LEMBO, AND G. FERRO, *Urology* **26,** 139 (1985).
3. H. M. NAGLER AND A. J. THOMAS, *Urol. Clin. North. Am.* **14,** 167 (1987).
4. R. E. GORDON, P. E. HANLEY, D. SHAW, D. C. GADIAN, G. K. RADDA, P. STYLES, P. J. BORE, AND L. CHAN, *Nature (London)* **287,** 736 (1980).
5. P. J. BORE, P. A. SEHR, L. CHAN, K. R. THULBORN, B. D. ROSS, AND G. K. RADDA, *Transplantation Proc.* **13,** 707 (1981).
6. R. A. ILES, A. N. STEVENS, AND J. R. GRIFFITHS, *Prog. MR Spectroscopy* **15,** 49 (1982).
7. B. CHANGE, S. ELEFF, AND J. S. LEIGH JR., *Proc. Natl. Acad. Sci. USA* **77,** 7430 (1980).
8. B. D. ROSS, G. K. RADDA, D. C. GADIAN, G. ROCKER, M. ESIRI, AND J. FALCONER-SMITH, *N. Engl. J. Med.* **304,** 1338 (1981).
9. D. C. GADIAN. "Nuclear Magnetic Resonance and Its Applications to Living Systems." Clarendon, Oxford, 1982.
10. J. J. H. ACKERMAN, T. H. GROVE, G. G. WONG, D. C. GADIAN, AND G. K. RADDA, *Nature (London)* **283,** 167 (1980).
11. A. P. KORETSKY, S. WANG, J. MURPHY-BOESCH, M. P. KLEIN, T. L. JAMES, AND M. W. WEINER, *Biochemistry* **80,** 7491 (1983).
12. E. ACHTEN, M. VAN CAUTEREN, A. WISANTO, R. LUYPAERT, AND M. OSTEAUX, *in* "Proceedings of the 7th Meeting of the SMRM, San Francisco, Book of Abstracts, 1988," p. 58.
13. W. M. CHEW, H. HRICAK, AND R. D. MCCLURE, *in* "Proceedings of the 7th Meeting of the SMRM, San Francisco, Book of Abstracts, 1988," p. 322.
14. J. VAN DER GROND, J. S. E. LAVEN, AND W. P. TH. M. MALI, *in* "Proceedings of the 8th Meeting of the SMRM, Amsterdam, Book of Abstracts, 1989," p. 577.
15. A. A. TZIKA, D. B. VIGNERON, H. HRICAK, B. A. KOGAN, M. E. MOSELEY, AND T. L. JAMES, *in* "Proceedings of the 7th Meeting of the SMRM, San Francisco, Book of Abstracts, 1988," p. 438.
16. P. N. BRETAN, D. B. VIGNERON, H. HRICAK, R. D. MCCLURE, T. S. BENEDICT, M. MOSELEY, E. A. TANAGHO, AND T. L. JAMES, *Radiology* **162,** 867 (1987).
17. J. W. PETTEGREW, S. J. KOPP, S. J. MINSHEW, T. GLONEK, J. M. FELIKSIK, J. P. TOW, AND M. M. COHEN, *J. Neuropathol. Exp. Neurol.* **46,** 419 (1987).
18. P. F. DALY, R. C. LYON, P. J. FAUSTINO, AND S. J. COHEN, *J. Biol. Chem.* **262,** 14,875 (1987).
19. R. W. MCGILVERY. "Biochemistry: A Fundamental Approach." Saunders, Philadelphia, 1970.
20. J. VAN DER GROND, D. SEIJKENS, AND W. P. TH. M. MALI. *Magn. Reson. Med.* **15,** 612 (1990).
21. H. D. SOSTMAN, I. M. ARMITAGE, AND J. J. FISCHER, *Magn. Reson. Imaging* **2,** 265 (1984).
22. O. A. C. PETROFF, J. W. PRICHARD, K. L. BEHAR, J. R. ALGER, J. A. DEN HOLLANDER, AND R. G. SHULMAN, *Neurology* **35,** 781 (1985).
23. E. P. KENNEDY AND S. B. WEISS, *J. Biol. Chem.* **222,** 193 (1956).
24. P. H. PRITCHARD AND D. E. VANCE, *Biochem. J.* **196,** 261 (1981).

25. G. CARPINELLI, F. PODO, M. DIVITO, E. PROIETTI, S. GESSANI, AND F. BELARDELLI, *FEBS* **176,** 88 (1984).
26. C. KENT, *Proc. Natl. Acad. Sci. USA* **76,** 4474 (1979).
27. R. SLEIGHT AND C. KENT, *J. Biol. Chem.* **255,** 10,644 (1980).
28. R. SLEIGHT AND C. KENT, *J. Biol. Chem.* **258,** 824 (1983).
29. R. SLEIGHT AND C. KENT, *J. Biol. Chem.* **258,** 831 (1983).
30. R. SLEIGHT AND C. KENT, *J. Biol. Chem.* **258,** 836 (1983).
31. S. L. PELECH AND D. E. VANCE, *Biochim. Biophys. Acta* **779,** 217 (1984).
32. R. H. HAMMERSTEDT AND H. A. LARDY, *J. Biol. Chem.* **258,** 8759 (1983).
33. J. K. BECKMAN, M. E. GRAY, AND J. G. CONIGLIO, *Biochim. Biophys. Acta* **530,** 367 (1978).
34. G. NAVON, R. NAVON, R. S. SHULMAN, AND T. YAMANE, *Proc. Natl. Acad. Sci. USA* **75,** 891 (1978).
35. C. T. BURT AND H. J. RIBOLOW, *Biochem. Med.* **31,** 20 (1984).
36. C. T. BURT, *Trends Biochem. Sci.* **10,** 404 (1985).
37. D. G. BROOKS, *Biochem. J.* **118,** 851 (1970).
38. M. D. SMITH, D. F. BABCOCK, AND H. A. LARDY, *Biol. Reprod.* **33,** 1029 (1985).
39. B. T. HINTON AND B. P. SETCHELL, *J. Reprod. Fertil.* **58,** 401 (1980).
40. H. RIBOLOW AND C. T. BURT, *in* "Magnetic Resonance of the Reproductive System," SLACK, Inc., p. 127, 1987.
41. A. S. LEVINE, N. FOSTER, AND B. BEAN, *Ann. N.Y. Acad. Sci.* **508,** 466 (1987).
42. W. S. ARRATA, C. T. BURT, AND S. CORDES, *Fertil. Steril.* **30,** 329 (1978).
43. M. AUROUX, N. N. Y. NAWAR, AND T. RIZKALLA, *Arch. Androl.* **14,** 155 (1985).
44. J. T. DAVIS, R. B. BRIDGES, AND J. G. CONIGLIO, *Biochem. J.* **98,** 342 (1966).
45. M. OSHIMA AND M. P. CARPENTER, *Biochim. Biophys. Acta* **152,** 479 (1968).
46. M. P. CARPENTER, *Biochim. Biophys. Acta* **231,** 52 (1971).
47. T. S. REDDY AND A. KHANNA, *Int. J. Vit. Nutr. Res.* **53,** 12 (1982).
48. M. NAKAMURA, B. JENSEN, AND O. S. PRIVETT, *Endocrinology* **82,** 137 (1968).
49. D. GAMBAL AND R. J. ACKERMAN, *Endocrinology* **80,** 231 (1967).
50. M. COBURN, T. WHEELER, AND L. I. LIPSHULTZ, *Urol. Clin. North Am.* **14,** 551 (1987).