Testicular Feminization of the Mouse

Paucity of Peroxisomes in Leydig Cells of the Testis

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The testes of mice with the X-linked testicular feminization (Tfm/y♀) mutation are very small and cryptorchid. The spermatogenesis in the adult Tfm/y♀ mouse testes is arrested, and the testosterone biosynthesis is significantly reduced due to deficiency of 17-ketosteroid reductase, the enzyme essential for the conversion of androstanedione to testosterone. In this study the distribution of peroxisomes in the Leydig cells of adult Tfm/y♀ mice was investigated because of the suggesttion that peroxisomes may participate in lipid metabolism and/or androgen biosynthesis in steroidogenic cells. Aldehyde-fixed testicular tissue of Tfm/y♀ mice was processed for the cytochemical localization of peroxisome catalase to facilitate identification of these organelles in the Leydig cells. Testes from Blo/y and CS^a strain normal adult mice served as controls. Testicular Leydig cells of normal adult Blo/y and CS^a mice contained abundant smooth endoplasmic reticulum (SER) in the form of complex interconnected tubules and double-walled membranous vesicles. Numerous peroxisomes, often in continuity with SER channels or in close association with lipid droplets, were observed in Leydig cells of normal males. In contrast, the peroxisomes in the Leydig cells of adult Tfm/y♀ mouse testes were either undiscernible or greatly reduced in number and size. SER in these cells was sparse, whereas mitochondria were numerous. In addition, abundant clusters of lipid droplets were encountered in a majority of Leydig cells of Tfm/y♀ mouse testes. Peroxisome and SER paucity in Leydig cells of Tfm/y♀ mice may be a reflection of reduced testosterone production. Whether excessive accumulation of lipid in the Leydig cells of Tfm/y♀ mouse testes is due to reduced utilization of cholesterol for the biosynthesis of testosterone or to impaired lipid metabolism due to reduction in peroxisome population in these cells remains to be ascertained. (Am J Pathol 1981, 103:96–104)

TESTICULAR FEMINIZATION MUTATION in man is a relatively rare inherited form of male pseudohermaphroditism.[1,2] The affected individual is phenotypically a normal female, endowed with a vagina and well-developed nipples, but lacking a uterus, uterine tubes, and ovaries.[2,3] Although these individuals with male genotype are cryptorchid, they lack androgen-dependent differentiation of the male genitalia due to end-organ unresponsiveness to testosterone.[2] Testicular feminization mutation (Tfm/y♀) has been discovered in rats[4,5] and mice[6] and appears homologous to the Tfm in human.[3] These animal models have already proved extremely useful in establishing the mode of inheritance of this disorder as X-linkage[3,7] and in elucidating the biochemical basis for the unresponsiveness of the target cells to androgens.[5,8,9] It now appears that the end-organ insensitivity to androgens leads to a lack of Leydig cell maturation and defective androgen biosynthesis in the testes of adult Tfm/y♀ animals.[10] The decline of testosterone production in postpubescent Tfm/y♀ testes in affected rats and mice has been attributed to the deficiency of 17-ketosteroid reductase.[9] The present study was undertaken to ascertain whether the reduction of testosterone biosynthesis and accumulation of lipid[10–12] in the testis of Tfm/y♀ animals is associated with alterations in peroxisome population in Leydig cell cytoplasm, since these organelles appear to play a role in lipid metabolism.[13–15] Furthermore, the identification of

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peroxisomes in the Leydig cells of normal rodent testes prompted Reddy and Svoboda[16,17] to suggest that peroxisomes, possibly through the regulation of NADPH levels, influence androgen biosynthesis and cholesterol metabolism, because the testicular microsomal hydroxylases, as well as Δ^4-reductases are NADPH-dependent. We now report that the peroxisomes in the Leydig cells of adult Tfm/y♀ mouse testes are greatly reduced in size and number. A preliminary account of these findings has been reported.[17]

Materials and Methods

Tfm/y♀ feminized mice and normal Blo/y male mice, 9–15 weeks old, were obtained from the colony maintained at the City of Hope National Medical Center, Duarte, California. Cs^a strain wild type normal male mice were derived from a colony maintained in our laboratory (JKR). Small segments of testes were fixed for 30 minutes by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at 4 C; these were then postfixed in OsO_4 and processed for electron microscopy. For the cytochemical procedure, tissue was fixed in glutaraldehyde for 4 hours, rinsed overnight in 0.1 M cacodylate buffer containing 0.2 M sucrose and incubated in the 3,3′-diaminobenzidine medium (DAB), pH 9.3, for 45–60 minutes at 37 C for the localization of the peroxidatic activity of peroxisomal catalase.[17,19] Controls consisted of incubations in the DAB medium containing 0.02 M aminotriazole, 0.01 M KCN, or 0.1 M sodium azide as described previously.[17,20] After incubation, the tissue was rinsed in buffer and postfixed for 1 hour in 2% OsO_4 in 0.1 M *s*-collidine buffer, pH 7.4, dehydrated in graded series of ethanol, and embedded in Epon. Thin sections were cut and examined either unstained or counterstained with lead hydroxide.

Results

The cryptorchid testes of adult Tfm/y♀ mice (Figure 1A) are small when compared with the testes of normal Blo/y (Figure 1B) or CS^a strain male mice. Histologically, the seminiferous tubules of Tfm/y♀ mouse testes are underdeveloped and contain mostly the Sertoli cells, spermatogonia, and an occasional primary spermatocyte. The interstitial tissue appeared prominent with numerous Leydig cells. These cells displayed abundant lipid in their cytoplasm (Figure 2).

The cytoplasm of testicular Leydig cells of the normal Blo/y mouse (Figure 3) or CS^a strain mouse contained several peroxisomes, which appeared as oval to spherical organelles with a homogeneous matrix. These organelles measured 0.05–0.5 μ in size and were scattered throughout the cytoplasm in close association with the smooth endoplasmic reticulum (SER) and lipid droplets (Figure 4). Examination of DAB-incubated testicular tissue from normal males showed that these organelles contained electron-opaque DAB reaction product indicative of the presence in them of peroxisomal marker enzyme catalase (Figure 4). In addition, the Leydig cells had an abundant SER in the form of complex interconnected tubules and focal areas of membranous whorls (Figure 4). Several foci of double membrane tubular vesicles were also noted in the Leydig cell cytoplasm of normal mouse testes.

In contrast, the cytoplasm of Leydig cells of Tfm/y♀ mouse testes contained very few or no peroxisomes when examined following DAB incubation (Figures 5–7). When present, the peroxisomes were discernible as very small (0.1 μ in diameter) structures containing flocculent DAB-positive reaction product (Figure 7). The SER in the Leydig cells of Tfm/y♀ mouse testes appeared greatly reduced in a majority of Leydig cells, particularly those containing numerous clusters of lipid droplets. Some Leydig cells in Tfm/y♀ mouse testes appeared ultrastructurally similar to those seen in normal mouse testes and contained appreciable amounts of SER. However, very few peroxisomes were discerned in such cells following DAB incubation (Figure 5). The cytoplasm of many Leydig cells was characterized by abundant aggregates of lipid droplets (Figure 6), sometimes in conjunction with densely packed mitochondria. The mitochondria in these Leydig cells displayed tubular cristae (Figures 6 and 7) characteristic of mitochondria in steroid-synthesizing cells. In mitochondrial-rich Leydig cells, peroxisomes either were absent or, when present were greatly reduced in size and number amid mitochondria and clusters of lipid droplets (Figure 7). The presence of DAB reaction product in the erythrocytes present in the intersubular vessels in the Tfm testes (Figures 5 and 6) indicates that the cytochemical staining conditions are optimal. The reaction product in the erythrocytes serves as positive internal control.

Discussion

This ultrastructural cytochemical study confirms the extent of overall degree of cytodifferentiation reported previously in the Leydig cells of testes in rats and mice with Tfm/y♀ .[10–12] The study of Blackburn et al[10] and Chung et al[11] on the Leydig cell ultrastructure of Tfm/y♀ mouse testes revealed that these

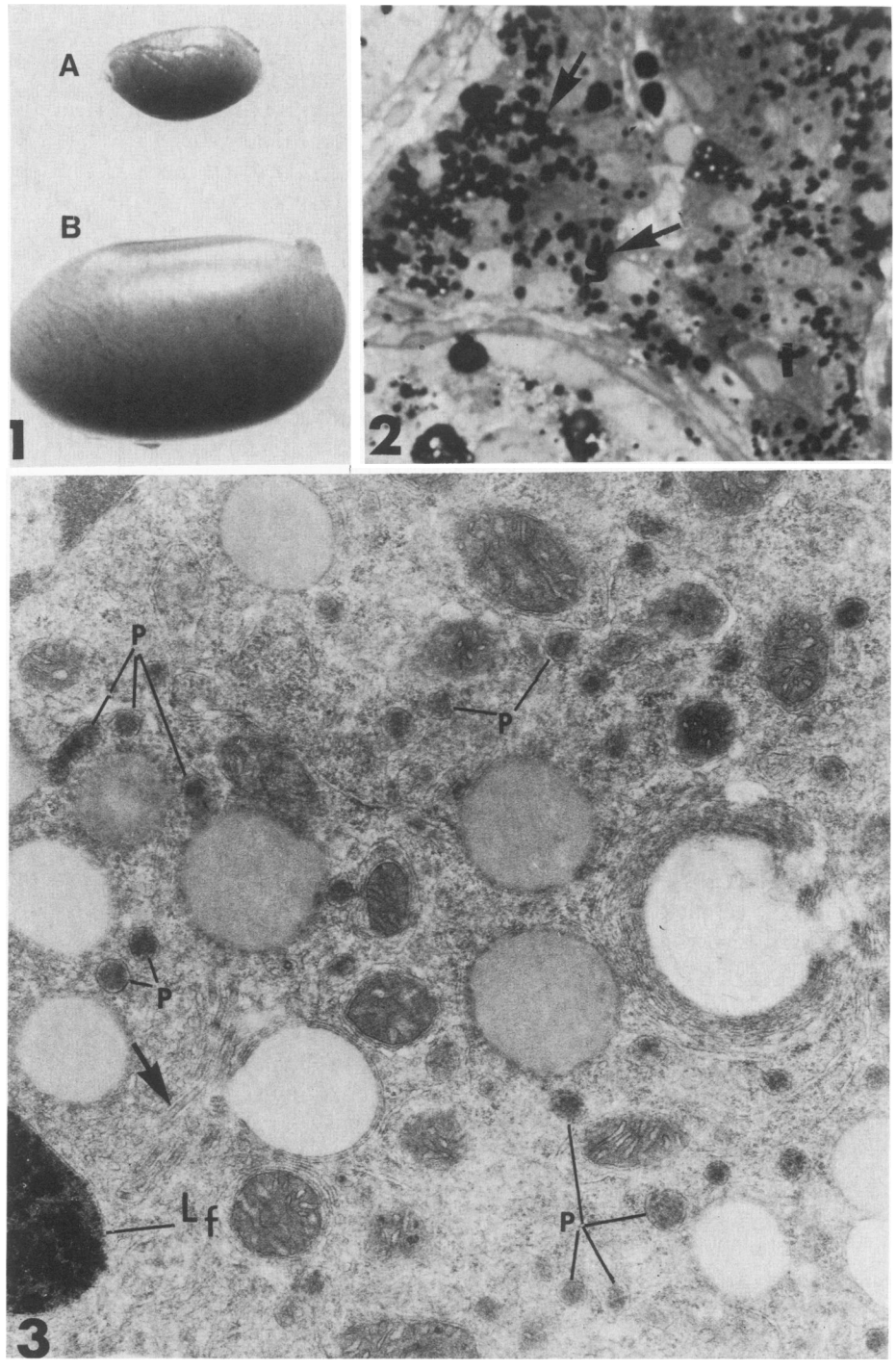

Figure 1—Comparison of testis size: **A**—Cryptorchid testis of adult Tfm/y♀ mouse. **B**—Testis of adult Blo/y normal mouse. **Figure 2**—Thick section of Epon-embedded testis of an adult Tfm/y♀ mouse. Note the presence of numerous lipid droplets in the cytoplasm of Leydig cells (*arrows*). (Toluidine blue, ×2200) **Figure 3**—A portion of the Leydig cell of normal adult Blo/y mouse testis reveals the presence of numerous single-membrane-limited organelles identified as peroxisomes (*P*). Note the close proximity of these organelles to lipid droplets. L_f, lipofuscin. *Arrow* points to double-walled vesicles (*arrow*). (Lead hydroxide, ×23,000)

cells contain increased quantities of lipid and mitochondria but relatively scant SER, thereby differing markedly from the appearance of Leydig cells of normal adult mouse testes.[21] We now show, using the DAB cytochemical procedure, that the Leydig cells of adult Tfm/y♀ mouse testes contain very few or no peroxisomes when compared with these organelles in normal mouse testicular Leydig cells.[17]

Earlier studies from our laboratory have identified peroxisomes in the Leydig cells of normal adult rat,

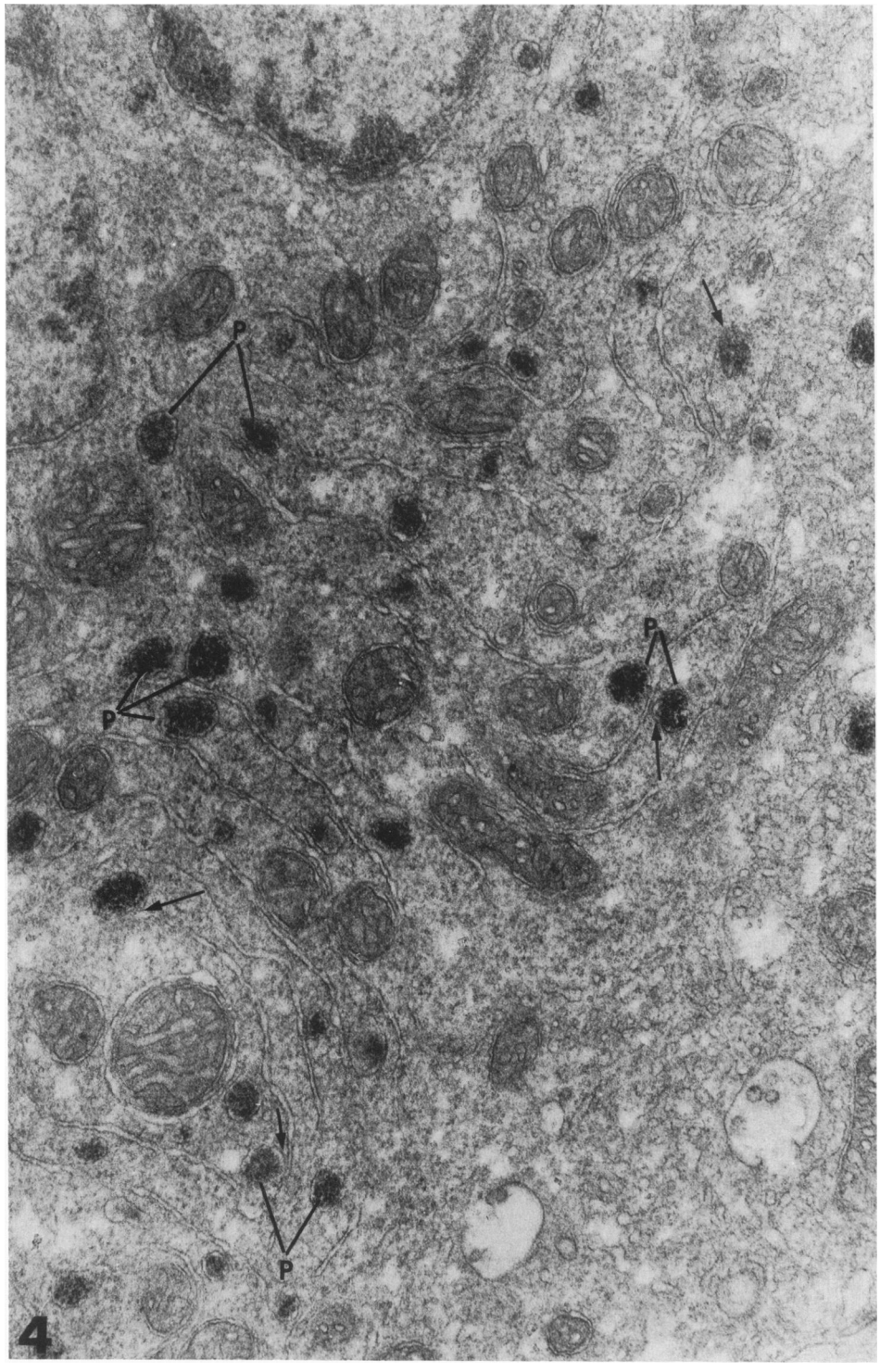

Figure 4—Testicular tissue of normal adult CS^{a} male mouse incubated for 45 minutes at 37 C in the DAB medium, pH 9.3. The DAB reaction product is seen in the peroxisomes (*P*). Note the close spatial arrangement of these organelles with the endoplasmic reticulum (*arrows*). (×32,000)

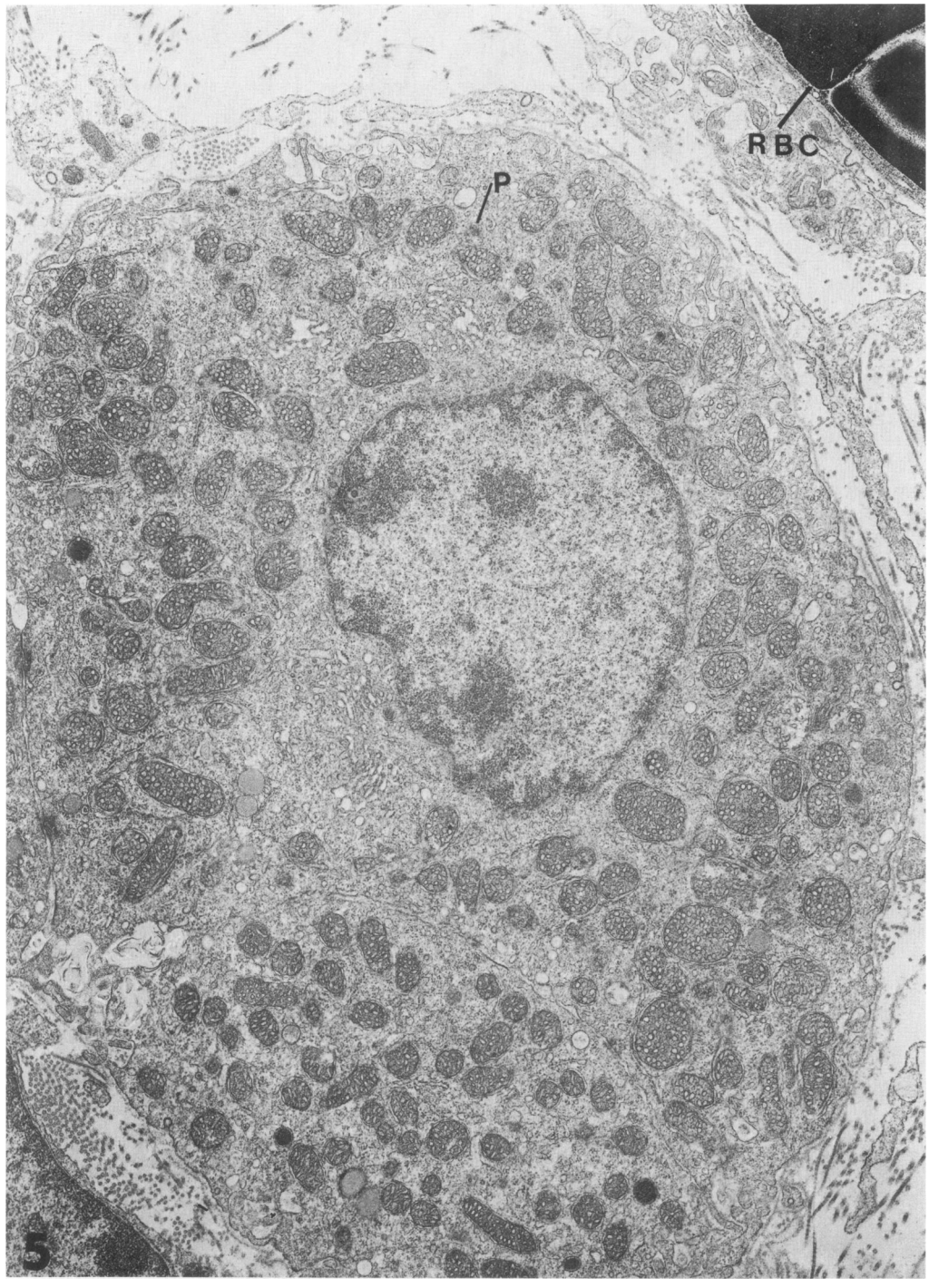

Figure 5—Tfm/y♀ mouse testis incubated in the DAB medium, pH 9.3, for 60 minutes at 37 C. The DAB-positive peroxisomes are not conspicuous in the cytoplasm of this Leydig cell (compare with Figure 4). An occasional small structure, with very weak DAB-positive material, is seen in the cytoplasm (*P*), which may represent a peroxisome. Note that the red blood cells (*RBC*) show an intense DAB reaction product due to the peroxidatic activity of hemoglobin. (× 24,500)

mouse, and guinea pig testes,[16,17] as well as in the testicular Leydig cell neoplasms of rats occurring either spontaneously[20] or after cadmium administration.[22] In the normal Leydig cells, these organelles measure 0.1–0.5 μ in diameter, are limited by a single membrane, and occur abundantly in close association with the SER and lipid.[17] In the neoplastic Leydig cells, peroxisomes are numerous, and many of these contain tubular inclusions of uncertain nature.[20,22] The presence of catalase in the matrix of

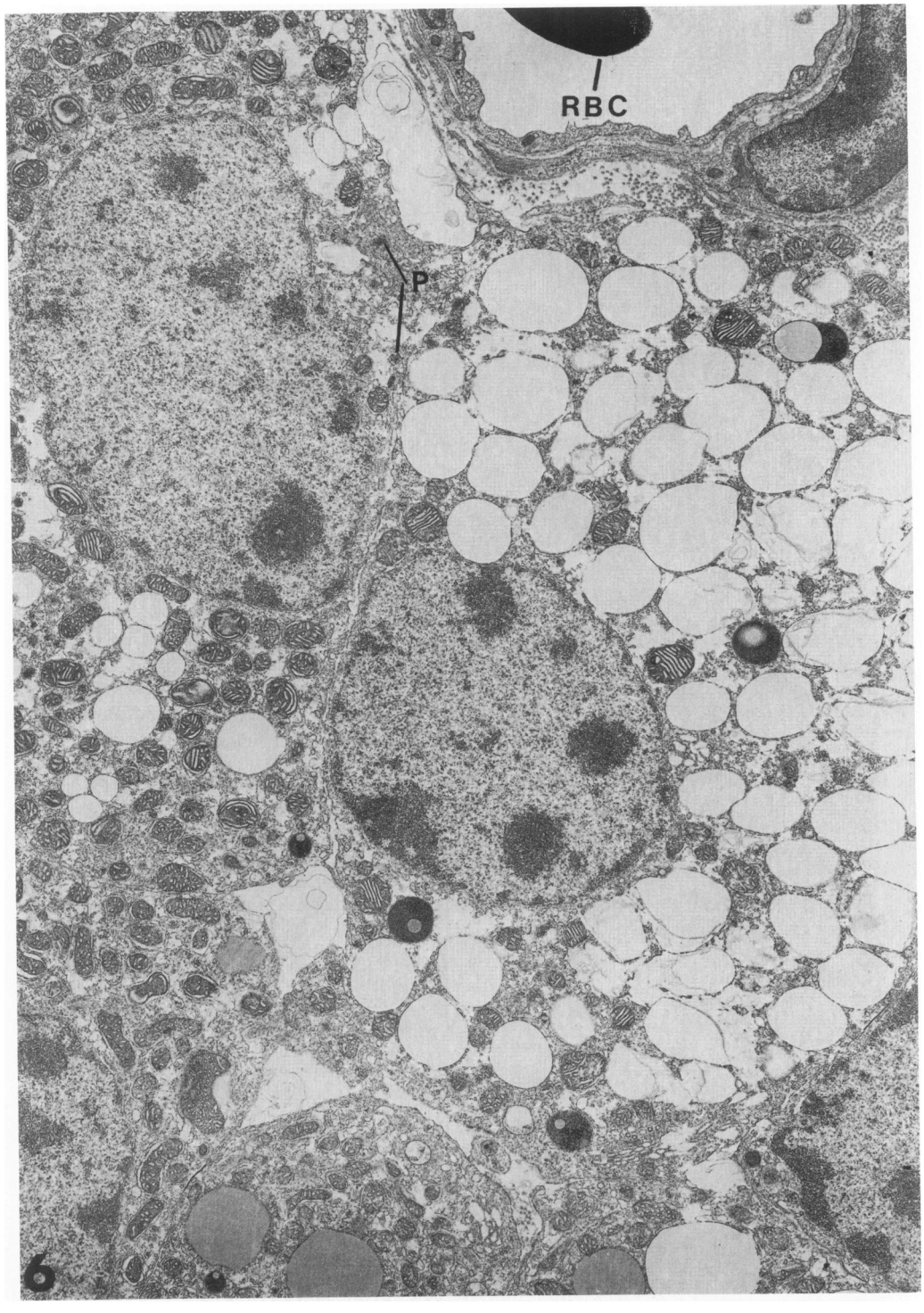

Figure 6—Tfm/y♂ mouse testis incubated in the DAB medium as in Figure 5. Note the reaction product in red blood cells (*RBC*). No DAB-positive organelles, suggestive of peroxisomes, are found in the cytoplasm of the Leydig cells, which contain abundant lipid. An occasional small single-membrane-limited structure, similar to an SER vesicle, can be identified as a peroxisome (*P*) with faint reaction product. (× 19,500)

peroxisomes facilitates the positive identification of these organelles by the DAB cytochemical procedure.[19] This cytochemical procedure has enabled, during the past decade, the recognition of peroxisomes as ubiquitous constituents in animal and plant cells.[23,24] In addition to catalase, these cytoplasmic organelles contain several hydrogen peroxide-generating oxidases, carnitine acetyltransferase, and enzymes involved in the β-oxidation of long-chain fatty acids.[25] Evidence now indicates that peroxisomes play an im-

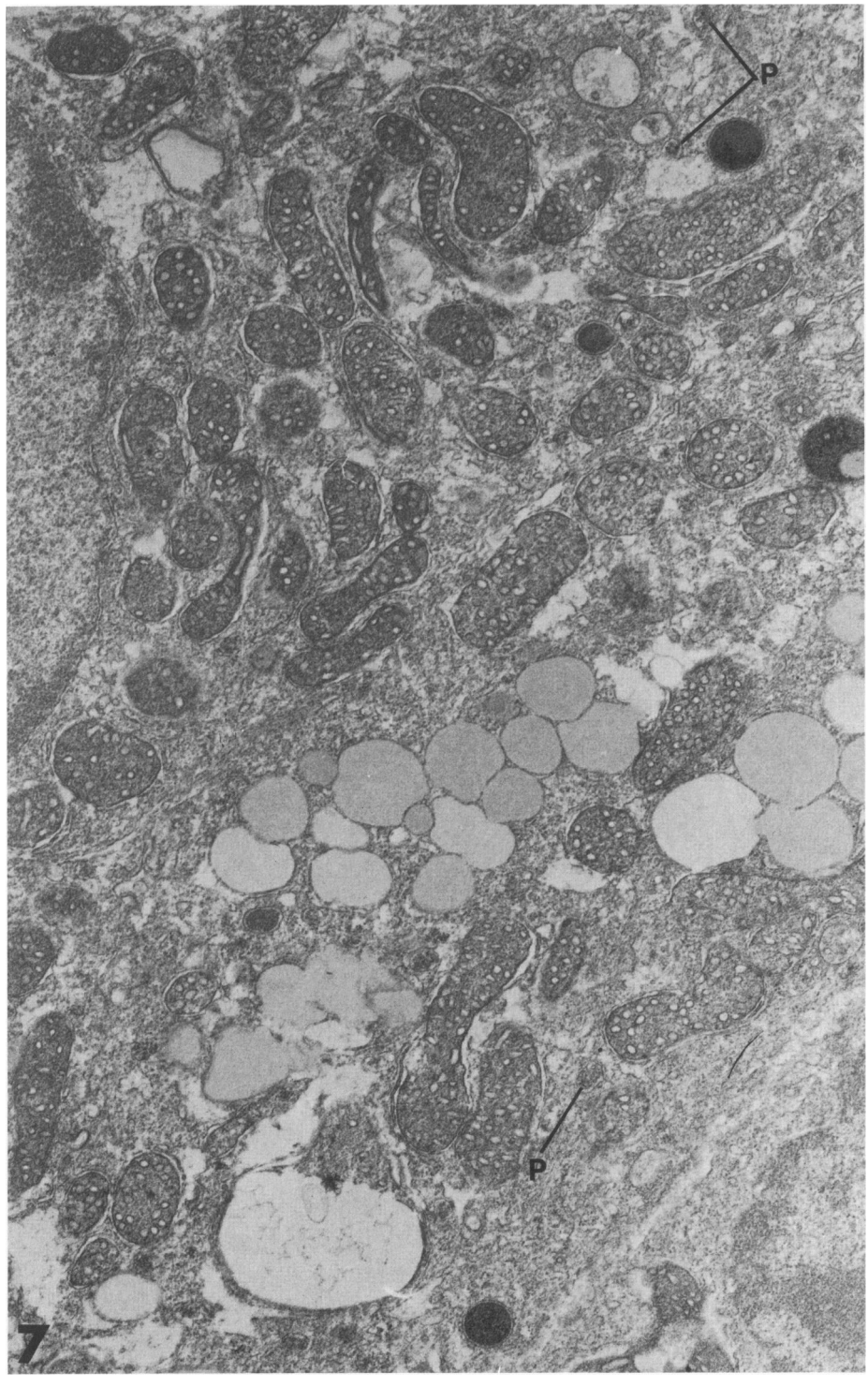

Figure 7—Tfm/y♀ mouse testis incubated in the DAB medium as in Figure 6. The Leydig cell contains many lipid droplets and scant SER. Note the presence of a few very small peroxisomes (*P*) showing DAB-positive material. (×31,000)

portant role in the lipid metabolism[14,15] and possibly in the development of liver tumors in rats treated for long periods with hypolipidemic peroxisome proliferators.[26] The presence of abundant peroxisomes in endocrine cells that utilize cholesterol for steroidogenesis[16,17,27,28] led to the speculation that these organelles in Leydig cells of normal testes and adrenal cortex may participate in androgen biosynthesis and cholesterol utilization.[17,28]

The significance of the paucity of peroxisomes in

the Leydig cells of adult Tfm/y♀ mouse testes is not clear. Tfm/y♀ mouse Leydig cells contain markedly reduced amount of tubular SER profiles.[10,11] The reduction in SER[10,30] may therefore have resulted in a concomitant reduction in peroxisome number in Tfm/y♀ mouse testes, since peroxisomes in many cell types, including those in the Leydig cells, are intimately associated with and interconnected to SER membranes.[17,20,23,24,29] Whether the reduction in Leydig cell peroxisome population merely reflects the intraabdominal (cryptorchid) location or is due to inherent decrease of 17-ketoseroid reductase activity and of androgen biosynthesis in Tfm/y♀ mouse testes needs to be investigated. The excessive accumulation of lipid in Tfm/y♀ mouse Leydig cells, may, however, be attributable to impaired lipid metabolism, possibly resulting from lack of adequate peroxisomes in these cells. Additional studies are required for us to determine the role of peroxisomal β-oxidation of fatty acids in the Leydig cell lipid metabolism and to ascertain whether aberrations in the peroxisomal population influence lipid accumulation. It should be noted that accumulation of lipid in various tissues is a prominent feature in cerebrohepato-renal syndrome (Zellweger's disease), a rare familial malady of infants in which peroxisomes cannot be found in the liver and kidney.[31] It is also pertinent that peroxisomes were not discerned by routine electron microscopy in the Leydig cell tumors occurring spontaneously in the testes of Tfm rats.[32] Likewise, testicular neoplasms of Tfm mice are characterized by abnormal accumulation of lipid in the neoplastic Leydig cells.[33] Whether this derangement of lipid metabolism in Leydig cell tumors of Tfm animals is due to drastic reduction of peroxisome population is not known. The absence or reduction of peroxisomes in the Tfm Leydig cell tumors of rats and mice should, however, be confirmed by the cytochemical localization of peroxisomal catalase.

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