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Spermatogenic Response to Vitamin A in Vitamin A Deficient Rats

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ABSTRACT

Male Holtzman rats were given a vitamin A deficient (VAD) diet from 21 days of age and retinoic acid after the onset of vitamin A deficiency. At 130 days of age, seminiferous tubules contain Sertoli cells, spermatogonia and preleptotene spermatocytes; serum LH and testosterone values are low normal, while FSH levels are high. Oral feeding of a single dose of 1 mg vitamin A (followed by a regular commercial rat pellet diet) causes reinitiation of spermatogenesis, although testosterone remains low and FSH does not return to normal for 60 days. After vitamin A treatment (PVA), kinetic characteristics based upon histologic criteria of the reinitiated spermatogenesis in VAD rats were normal. Pachytene spermatocytes can be seen by Day 14 PVA and spermatids by Day 24 PVA; elongation of spermatids was completed by Day 31 PVA and spermatozoa were formed by Day 41. Quantitatively, sperm production remained below normal at 150 days PVA, although epididymal sperm counts had continued to increase PVA to 50% of that in mature controls. The qualitative normalcy of spermatogenesis in PVA-VAD rats was demonstrated by fertility with normal litter size. Thus, VAD causes reversible germ cell depletion. Reinitated spermatogenesis in the VAD rat provides a kinetically normal, in vivo system in which functionally normal spermatozoa are produced and in which it may be possible to study the biochemical and morphological events of specific stages of spermatogenesis.

INTRODUCTION

Vitamin A deficiency in male rats causes degeneration or loss of germ cells which is characterized by seminiferous tubules containing only Sertoli cells, spermatogonia and a small number of spermatocytes (Thompson et al., 1964; Howell et al., 1963). Although normal growth can be maintained by retinoic acid in rats on a vitamin A deficient diet, maintenance or reinitiation of spermatogenesis in these rats requires either retinol or its aldehyde derivative, retinyl acetate (Howell et al., 1963; Thompson et al., 1964; Coward et al., 1969; Ahluwalia and Bieri, 1971b). The germinal epithelium shares this unique biochemical requirement only with the pigment epithelium of the retina (Dowling and Wald, 1960).

Vitamin A deficient rats were also found to have small seminal vesicles (Mason, 1933; Mayer and Truant, 1949; Thompson et al., 1964) and low circulating androgen levels (Howell et al., 1963; Krueger et al., 1974; Rich and de Kretser, 1977). However, failure to reinitiate spermatogenesis in these rats with hormone therapy (Meyer and Truant, 1949; Mayer and Goddard, 1951; Ahluwalia and Bieri, 1971a) provides evidence that the requirement of the germinal epithelium for retinol is not mediated by the testosterone deficiency. Although retinol-induced regeneration of spermatogenesis in rats maintained on retinoic acid supplement for a prolonged period of time has been reported (Thompson et al., 1964), the time course and the extent of the regeneration, the normalcy of the spermatozoa so produced and the reproductive performance of the retinol fed vitamin A deficient rats have not been studied.

Recently, Krueger et al. (1974) reported that maximal germ cell loss occurs by 130 days of age if weanling rats are given a vitamin A deficient diet, later supplemented by retinoic acid to maintain normal body growth. Although luteinizing hormone and testosterone levels were low, follicle stimulating hormone was normal and an intact pituitary-testicular axis was demonstrated by the elevation of LH and FSH following castration. In addition, the testosterone levels observed should be sufficient

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to maintain spermatogenesis, since less than 10% of normal intratesticular testosterone concentration was found to be able to maintain normal spermatogenesis (Cunningham and Huckins, 1978).

Thus, we attempted to determine the normalcy of spermatogenesis following vitamin A treatment of vitamin A deficient rats. If it can be shown that the regrowth of the germinal epithelium proceeds in an orderly, predictable fashion and if the resulting spermatozoa are functionally normal, then post-vitamin A (PVA) spermatogenesis in vitamin A deficient (VAD) rats might serve as a useful in vivo system in which the biochemical kinetics of germ cell development could be studied. We present herein the morphological and physiological characteristics of germ cell repletion in PVA-VAD male rats.

MATERIALS AND METHODS

Chemicals

Radioactive materials were purchased from New England Nuclear and used without further purification; Ilford nuclear emulsion (K5) was purchased from Ilford Nuclear Research (Essex, England). Ingredients for preparing vitamin A deficient diet were purchased from I.C.N. Life Science Group, Cleveland, OH. Reagents used for radioimmunoassay of LH and FSH were provided by Rat Pituitary Distribution program NIAMDD, NIH. Other general chemicals, all reagent grade, were purchased from Fisher Scientific Co.

Preparation of Vitamin A Deficient (VAD) Rats

Weanling male Sprague-Dawley rats Holtzman Co., Madison, WI), 19-21 days of age, were caged in pairs in an air conditioned, light controlled animal room used solely for vitamin A deficiency studies. They were given water ad libitum and the modified vitamin A deficient diet of Muto et al. (1972), using vitamin K instead of menadione. Animals were weighed twice weekly. Retinoic acid (5 mg/kg) was added to the VAD diet when the linear weight gain ceased as a result of complete depletion of vitamin A stores (Thompson et al., 1964; Muto et al., 1972). At 130 days of age, rats were given 1 mg vitamin A orally and, thereafter, fed a regular commercial rat pellet.

Histology and Radioautography

Two to 6 animals were sacrificed at intervals of 4-6 days after vitamin A administration in an effort to determine the sequential changes during spermatogenic regeneration. In selected VAD animals, 25 μCi [^{3}H]-thymidine (sp act 41.6 mCi/mg) were injected in 0.1 ml Ringer's solution intratesticularly under light ether anesthesia 24 h prior to sacrifice. About one-quarter of each testis was fixed in Bouin's solution. Paraffin embedded tissues were sectioned at 4 μm and stained with hematoxylin and eosin (H-E), or periodic acid Schiff reagent (PAS-H), according to the method of Preece (1972). Radioautography of testicular tissues was performed according to the method of Kopriwa and Leblond (1962). Deparaffined slides were dipped in 50% Ilford nuclear emulsion (K5) at 45° C. Radioautographs were developed with Kodak D-19 after 3-4 weeks of exposure. Identification of different types of spermatogonia was based upon criteria of Leblond and Clermont (1952). Spermatids were identified by the PAS-positive acrosomal material (Leblond and Clermont, 1952). Tubular diameter measurements were made in at least 20 cross sections of seminiferous tubules with circular circumference from 2-7 animals at each time PVA.

Reproductive Performance Test

Groups of VAD rats were maintained for various periods of time up to 150 days PVA. After 60, 80, 100 and 150 days PVA, rats were housed with 2 cycling females for 10 days. Thereafter, any pregnant females were followed to term to assess litter size. PVA-VAD males were sacrificed at 60, 80, 100 or 150 days PVA. Epididymal sperm counts were determined by the method of Dyson and Orgebin-Crist (1973). Three rats were maintained on 3 μg vitamin A daily, instead of retinoic acid, from the onset of vitamin A deficiency to maintain spermatogenesis (Coward et al., 1969). Fertility was tested at 7 months of age and the rats were sacrificed to determine epididymal sperm counts.

Hormone Assay

Before animals were sacrificed, blood samples were taken under light ether anesthesia through heart puncture or from the retroorbital sinus. Blood samples were kept at 4° C overnight and serum was collected after centrifugation.

Radioimmunoassay of plasma gonadotropins was performed using reagents provided by NIAMDD, Rat Pituitary Hormone Distribution Program; NIAMDD-Rat FSH-RP-1, Rat-FSH-I-3, Anti-Rat FSH-S-6 and NIAMDD-rat-LH-RP-1, Rat-LH-I-4, Anti-Rat-LH-S-3 and -S-4 were used for FSH and LH assay, respectively. Hormones were iodinated with ^{125}I using the chloramine-T method (Greenwood et al., 1963). Iodinated hormones were purified through a Bio-Gel P-60 column. Intraassay coefficient of variation for both FSH and LH assays was <10%. RIA data from each experimental group in which data are compared were obtained from a single assay.

Testosterone assay was performed in the laboratory of Dr. Inge Dyrenfurth using anti-testosterone-3-oxime-HSA-S741 #7 antibody. The inter- and intraassay coefficiency of variation for testosterone assay was 13.5% and 9.9%, respectively. Testosterone was determined in equal volume pools from animals in each experimental group and from age matched normal males to obtain the volume of serum required for duplicate assays.

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Statistics

Student's t test was employed to compare the mean of hormonal measurements between VAD rats and normal rats and linear regression analysis was used in measurements of seminiferous tubule diameter, according to Dixon and Massey (1969).

RESULTS

Growth Characteristics

Figure 1 shows the growth curve of rats grown on a vitamin A deficient diet. Weight gain of these animals increased linearly until 9-10 weeks of age. At this time, a plateau, followed by a decline of body weight of some animals, signaled the depletion of liver and total body vitamin A stores. Weight gain again became linear with supplementation of 5 mg/kg retinoic acid to the VAD diet. Rats at 130 days of age attained weights similar to those of age-matched normal rats.

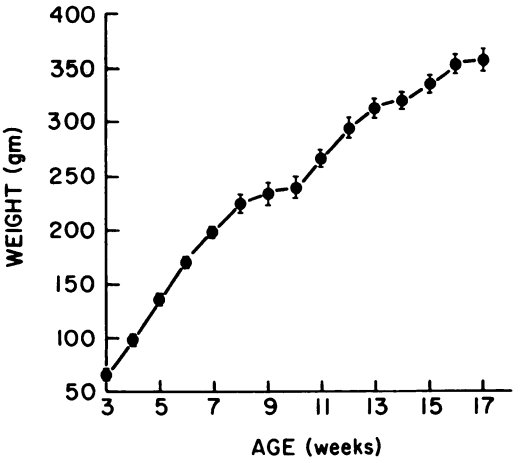

FIG. 1. Growth curve of vitamin A deficient (VAD) rats, expressed as mean ± SEM. Each point represents the mean value of 44 rats.

Testicular Histology

At the age of 130 days, the seminiferous tubules of VAD rats contained a markedly reduced number of germ cells (Fig. 2A); spermatogenesis did not proceed beyond early meiotic prophase. Some tubules contained predominantly Sertoli cells, whereas in others, 1 or 2 layers of germ cells were found in the basal compartment (Fig. 2B). Spermatogonia of all types and germ cells, the nuclear morphology of which resembled preleptotene spermatocytes, were found in the basal compartment of some tubules (Fig. 3). In other tubules, located on the adluminal side of Sertoli cell nuclei, atypical germ cell nuclei or degenerating nuclei which were labeled with [^{3}H]-thymidine 24 h prior to sacrifice (Fig. 4A,B) could be found. Prophase spermatogonia, also labeled with [^{3}H]-thymidine could be seen contiguous to preleptotene spermatocytes (Fig. 4C). In addition, spermatogonial mitoses were frequently observed (Fig. 4D).

Ten days PVA, the germinal epithelium became better organized and meiotic prophase had proceeded as far as pachytene spermatocytes (Fig. 5A). By Day 14 PVA, late pachytene spermatocytes were observed (Fig. 5B). By Day 24 PVA, spermatids appeared in the tubules (Fig. 5C) and PAS staining revealed that step 7-8 spermatids were present (Fig. 5D). Based on the averages of 2-4 animals at each time point and at least 100 observations/animal, PAS positive material was present in 45% of the tubules by Day 24 PVA, 74% by Day 37 PVA and 88% by Day 53 PVA. Elongation of sperm heads began by Day 27 PVA and was complete by Day 31 PVA (Fig. 6A). By Day 37, late spermatids appeared near the center of the tubule (Fig. 6B). Spermatozoa could be seen in the center of the seminiferous tubules by Day 41 PVA, which was earlier than the 56.3 days required for A_1 spermatogonia to become testicular spermatozoa (Huckins, 1978). By Day 62 PVA, cellular associations within most seminiferous tubules (Fig. 6C) were similar to those in normal rats (Fig. 6D). However, spermatogenesis was incomplete in 10-15% of the tubules, with occasional tubules containing only Sertoli cells.

Seminiferous Tubule Diameter (STD)

The diameter of seminiferous tubules of VAD rats at age 130-140 days was 130 ± 2.38 μm (mean ± SEM, n = 7 rats, n = 140 observations). This was significantly smaller than that of 4-month-old normal males (236 ± 4.70 μm), n = 2 rats, n = 40 observations). After vitamin A treatment, the increase in STD from Days 0-51 was highly significant ($t = 5.75$, $P<0.001$, Fig. 7). The data are also consistent with a triphasic increase. A significant increase in STD occurred between Day 0 and Day 8 PVA ($t = 3.51$, $P<0.01$), no change occurred between Day 8 and Day 37 and an additional increase was observed between Day 37 and Day 51 PVA ($t = 2.35$, $P<0.05$). The initial incrase in STD cannot be accounted for by the increase in cell

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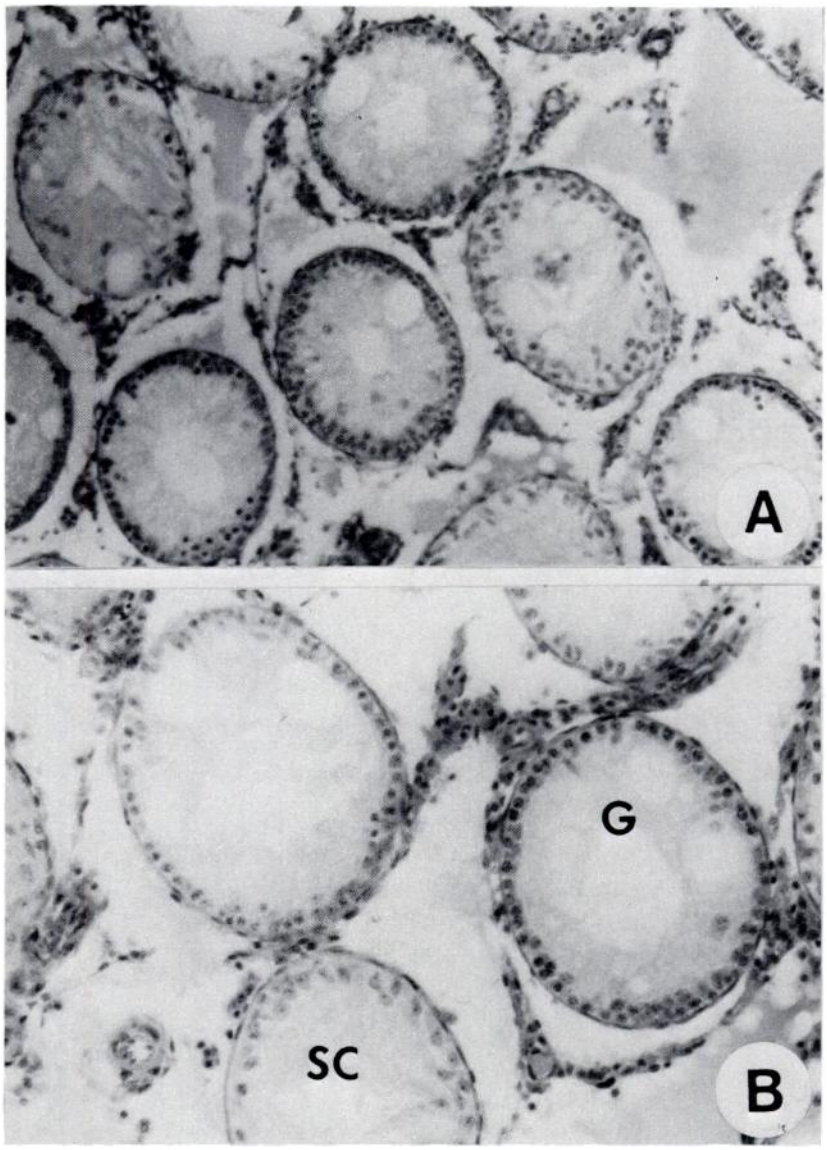

FIG. 2. Photomicrographs of testicular section of VAD rats at age 130 days (H-E). A) Seminiferous tubules relatively empty. X 37.5) B) Tubules containing mainly Sertoli cells (SC) or germ cells (G). X 60.

population and the mean diameter at 62 days remained significantly below normal.

Endocrine Status of VAD and PVA-VAD Rats

In 2 of the 3 experiments, LH levels in VAD rats were lower than those in age-matched controls (experiments 2 and 3; Fig. 8), although the differences were statistically significant ($P<0.01$) only in experiment 3. In experiment 1, LH levels of VAD rats were similar to those

FIG. 3. Photomicrographs of testicular section of VAD rats at age 130 days (A, C, H-E stained) and of normal rats (B, D, PAS-H stained). Sections of tubules in VAD rats cannot be staged due to disorganization of germinal epithelium. A) Tubules from VAD rat contain germ cells with nuclei resembling preleptotene spermatocytes (arrowheads). X 216.B) Preleptotene spermatocytes (arrowheads) in stage 1 (Huckins, 1978) of normal rat testis. X 216. Tubules from VAD rat which contain type B spermatogonia (arrowheads). X 216. D) Type B spermatogonia (arrowheads) in stage 6 (Huckins, 1978) of normal rat testis. X 216.

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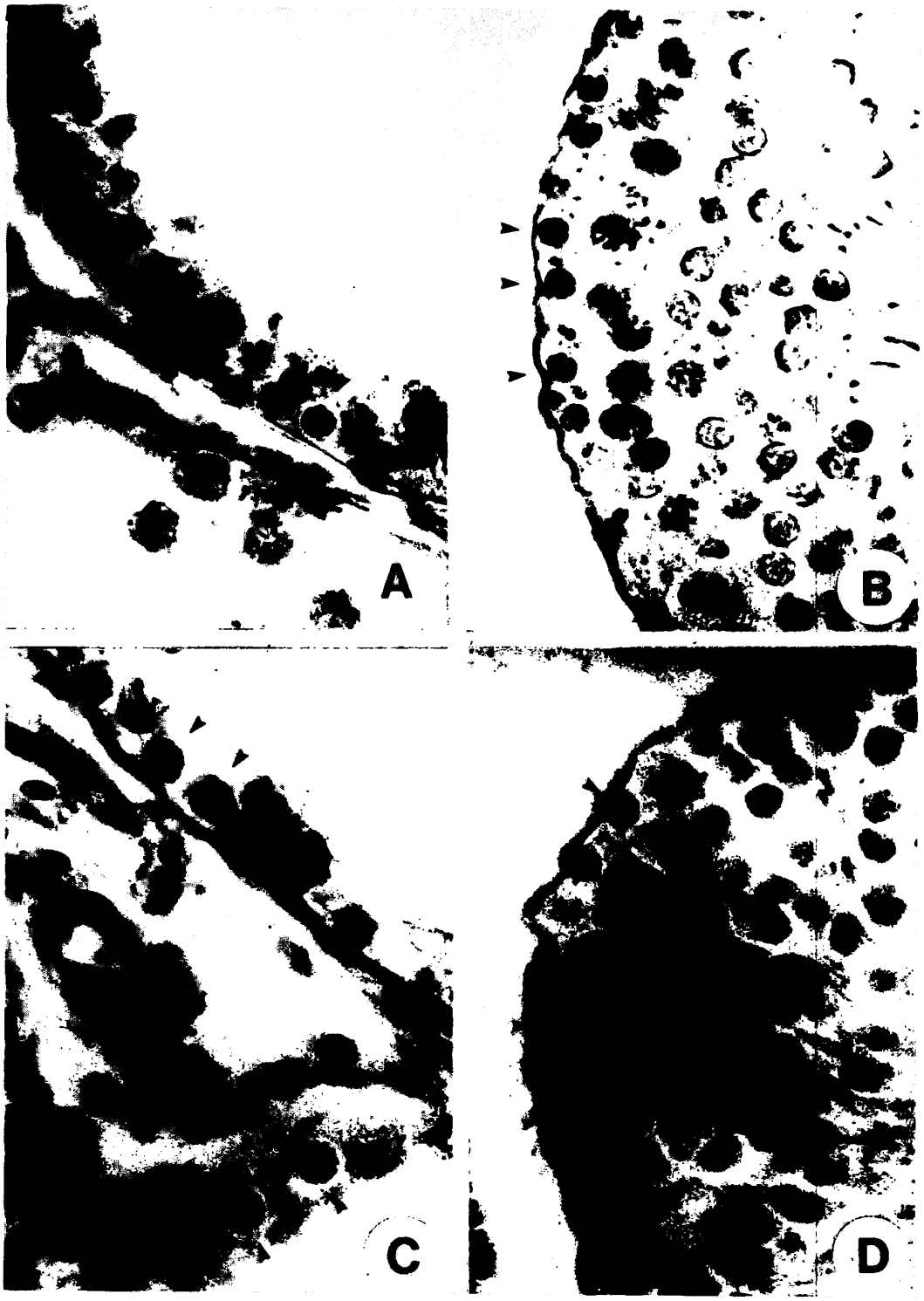

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assayed with the same reagents 6 months later. However, due to an experimental error, only 2 of 6 normal rats were assayed. After vitamin treatment, serum LH concentration increased significantly ($P<0.05$) by Day 10 PVA (Table 1). It reached the highest value on Day 20 PVA ($P<0.01$ vs normal control) and declined toward the base value by Day 50 PVA.

Serum testosterone level of VAD rats and age-matched normal males are shown in Fig. 9. Each dot represents a pool of sera obtained from 4-10 animals from all 3 experiments, assayed in a single assay. Testosterone levels in VAD rats were significantly lower ($P<0.01$) than those obtained from normal males. During the 60 days following vitamin A replacement, serum testosterone levels remained lower than those of normal males (Table 1).

Figure 10 shows the serum FSH level of VAD rats and age-matched normal males. In all 3 groups of VAD rats, serum FSH levels were significantly higher ($P<0.01$) than those of normal rats. After vitamin A treatment, serum FSH levels became significantly higher ($P<0.10$) than those of VAD rats by Day 10 PVA (Table 1), remained elevated until at least Day 50 PVA and then declined to normal by Day 60 PVA.

Reproductive Performance

Data summarizing the reproductive performance and epididymal sperm counts of PVA-VAD rats are given in Table 2. None of the 10 animals tested at 60 days PVA (group A) produced offspring. By 100 days PVA (group C), 3 of the 7 rats tested produced live offspring with a smaller than normal litter size, in contrast to the normal litter size in those rats (group E) which received 3 μg vitamin A daily after the onset of vitamin A deficiency at 9-10 weeks of age. Epididymal sperm counts of group C were 40 times higher than those of rats sacrificed 80 days PVA (group B). However, epididymal sperm counts remained only one-third the value of those of group E. By 150 days PVA (group D), 3 of 5 males tested produced normal sized litters and normal offspring, in spite of sperm count that were only one-half of those in group E animals and in normal rats.

DISCUSSION

Vitamin A alcohol, or retinol, is required for normal growth and function of mammalian tissues. This requirement for retinol can be replaced by retinoic acid in most tissues (Moore, 1957). However, as in the retina, there is a specific requirement for retinol in the testis for maintenance of normal spermatogenesis (Howell et al., 1963; Coward et al., 1969; Thompson et al., 1964; Ahluwalia and Bieri, 1971b). This study confirms that vitamin A deficiency is associated with a marked depletion of germ cells. It should be noted that spermatogonial division persists in the absence of vitamin A, although no quantitative comparison to that in normal rats was made. Nevertheless, mitotic figures among spermatogonia are frequently noted and preleptotene spermatocytes are found. Although a reduction in the number of spermatocytes had been reported in vitamin A deficient (VAD) rats (Mitranond et al., 1979; Thompson et al., 1964; Howell et al., 1963), published reports did not include observations that would permit identification of the specific stages of meiotic prophase present. In contrast, Krueger et al. (1974) reported that only Sertoli cells and spermatogonia remained in the tubules of VAD rats at 130 days of age. Results of the present study demonstrate that type B spermatogonia divide to form preleptotene spermatocytes, the latter being frequently observed throughout the testis of the VAD animal. In the absence of normal meiosis beyond preleptotene spermatocytes, one can conclude that normal progession of meiotic prophase cannot be achieved without vitamin A alcohol (retinol).

Atypical germ cell nuclei, located on the adluminal side of Sertoli cell nuclei, can also be found in tubules. The origin of these cells is not clear. The fact that they are labeled with [^{3}H]-thymidine 24 h prior to sacrifice indicates that they were derived from cells that had the ability to enter S-phase. Their location and

FIG. 4. Photomicrographs of portions of seminiferous tubules of VAD rats (H-E). A) Atypical nuclei (arrowheads) located at adluminal side of the Sertoli cell nuclei (SC) and dividing spermatogonium (Sg). X 346. B) Radioautograph of atypical nuclei (arrowheads) 24 h after intratesticular injection of [H^3]-thymidine. X 346. C) Radioautograph of spermatogonial prophase (arrowheads) 24 h after intratesticular injection of [H^3]-thymidine in proximity to series of preleptotone nuclei (PL). X 346. D) Spermatogonial mitotic figures (arrowheads). X 218.

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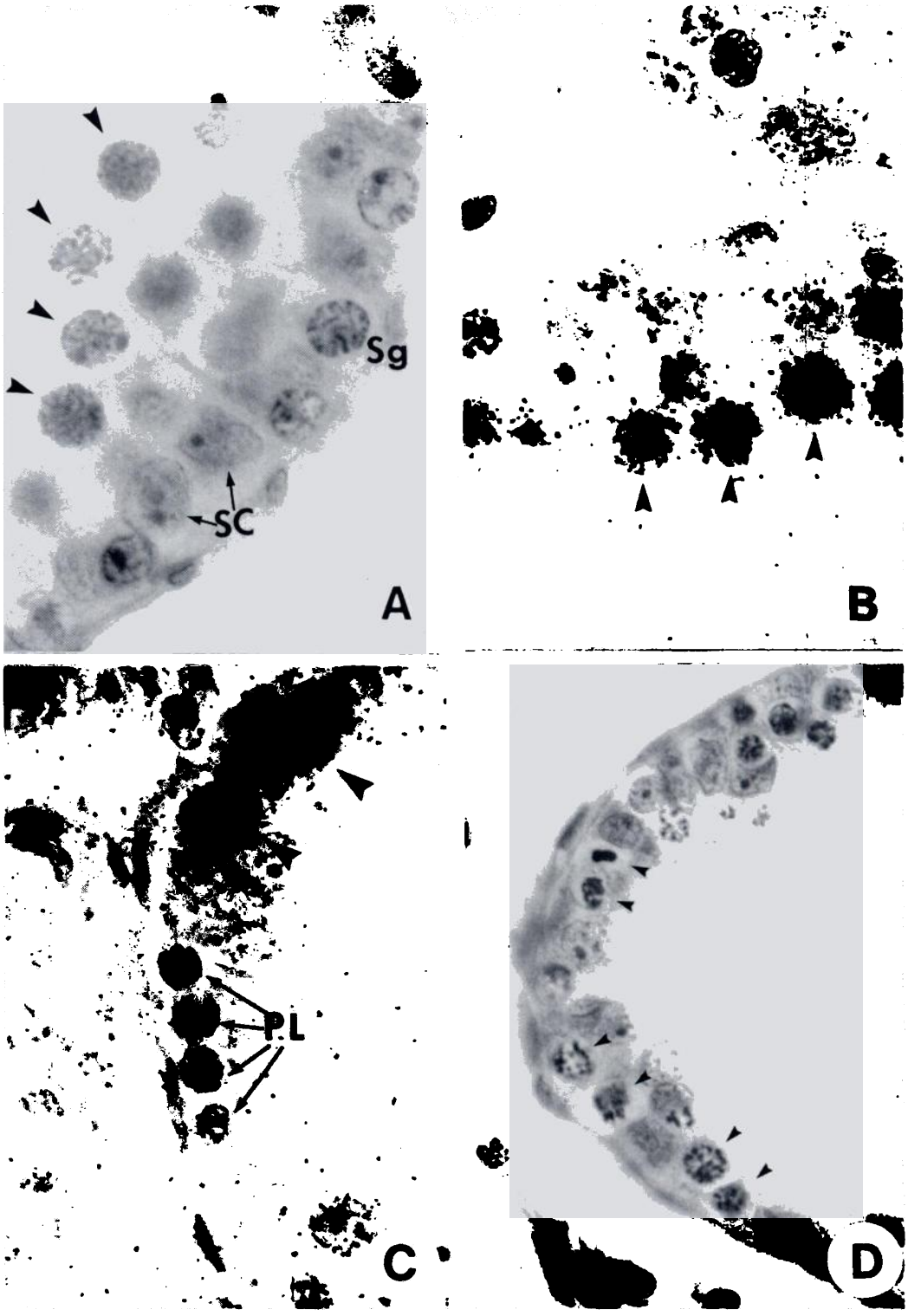

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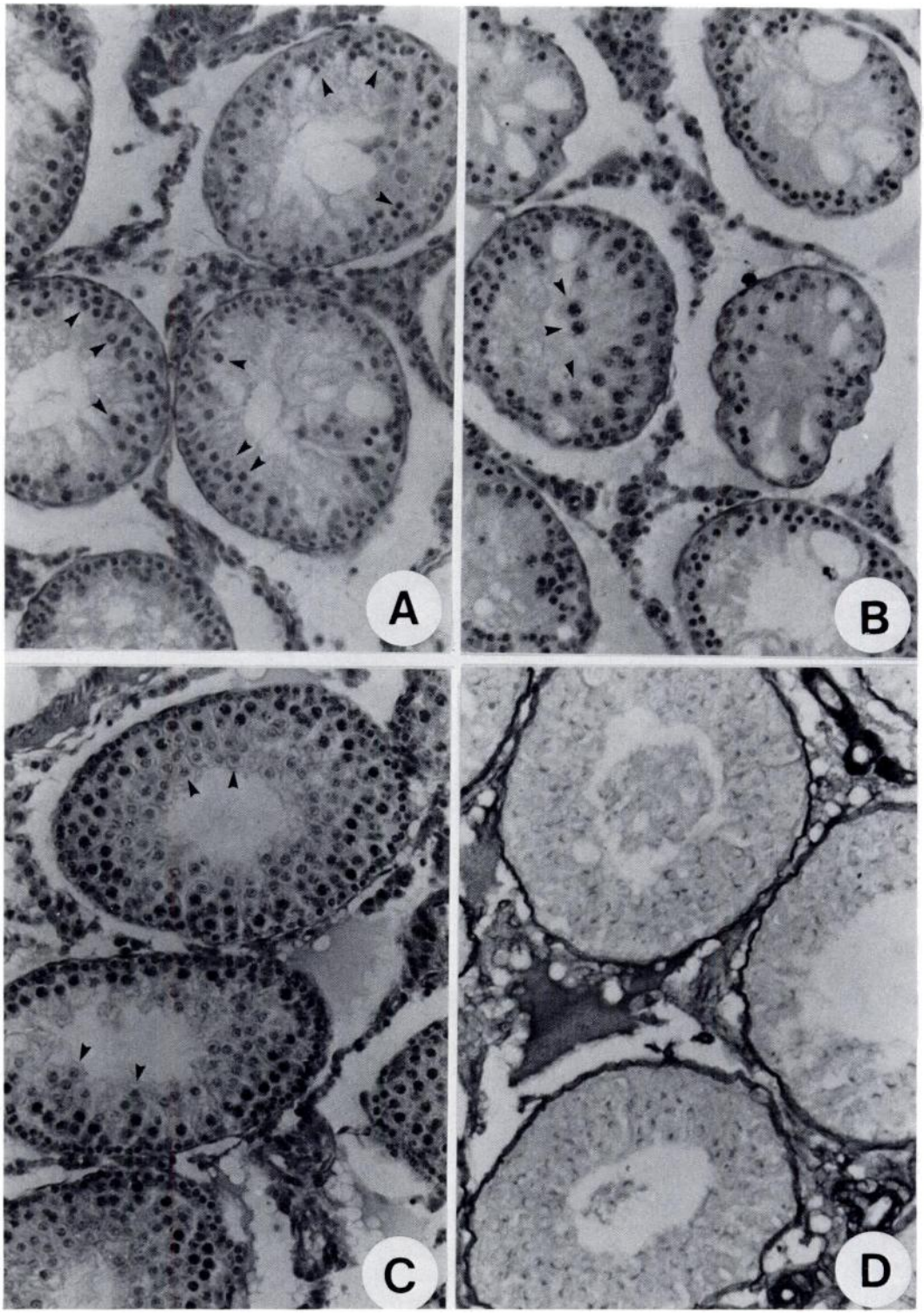

FIG. 5. Photomicrograph of testicular section of PVA-VAD rats (A-C, H-E stained; D, PAS stained). X 54. A) 10 days PVA, showing early pachytene spermatocytes (arrowheads). B) 14 days PVA, showing late pachytene spermatocytes (arrowheads). C) 24 days PVA, showing early spermatid (arrowheads). D) 24 days PVA, showing PAS positive acrosomal material in step 7-9 spermatids.

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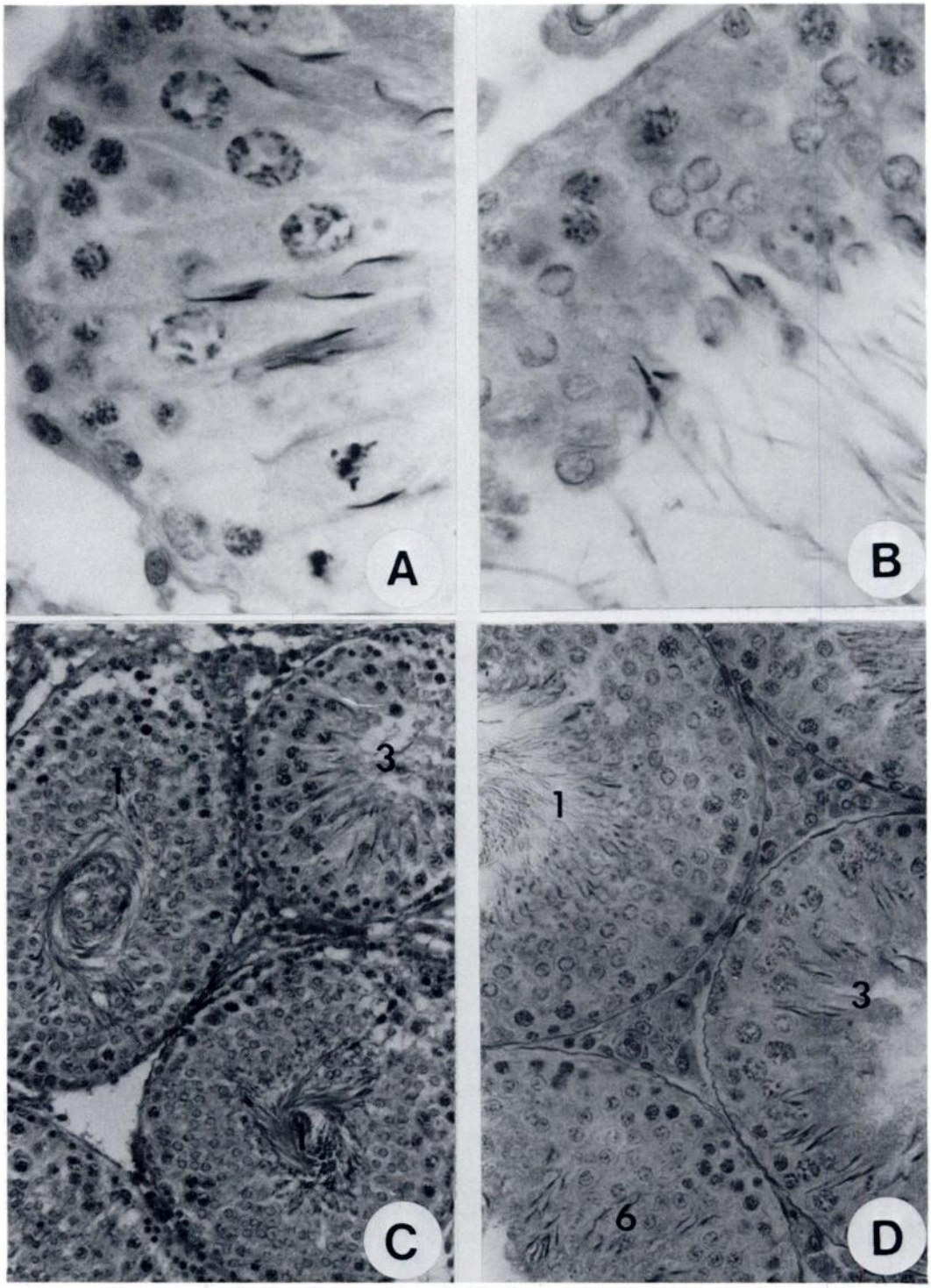

FIG. 6. Photomicrograph of testicular section of PVA-VAD rats (H-E stained). A) 31 days PVA, showing completion of the elongation of sperm head. × 220. B) 37 days PVA, showing step 18 spermatids in stage 6 of seminiferous epithelial cycle (Huckins, 1978). × 220. C) 62 days PVA, showing normal appearance of seminiferous tubules with identifiable stage of the germinal epithelium; number inside the tubules indicates the stage of seminiferous epithelial cycle (Huckins, 1978). × 56. D) Testicular tubules of a normal rat; number inside the tubules indicates the stage of seminiferous epithelial cycle (Huckins, 1978). × 56.

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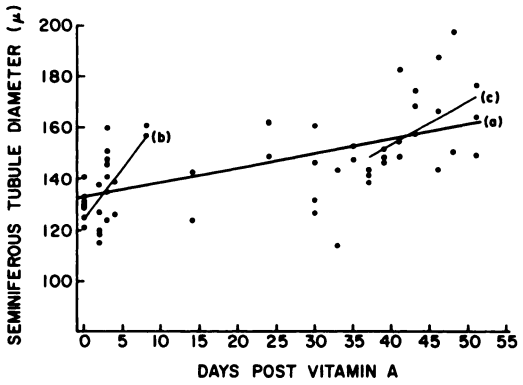

FIG. 7. Changes of seminiferous tubule diameter (μ) in PVA-VAD rats. Each point represents the average of 20 observations in 1 animal. Data were analyzed sequentially using linear regression. (a) The overall increase from Day 0 to Day 51 PVA was significant, ($y = 0.6056X + 132.27$, $r = 0.61$, $P<0.001$). (b) Regression analysis of data between Day 0 and Day 8 PVA reveals a significant correlation with a steeper slope, although the variance is greater, ($y = 3.923X + 124.42$, $r = 0.61$, $P<0.01$). (c) Changes between Day 37 and Day 51 PVA are also significant, ($y = 1.776X + 83.36$, $r = 0.48$, $P<0.01$).

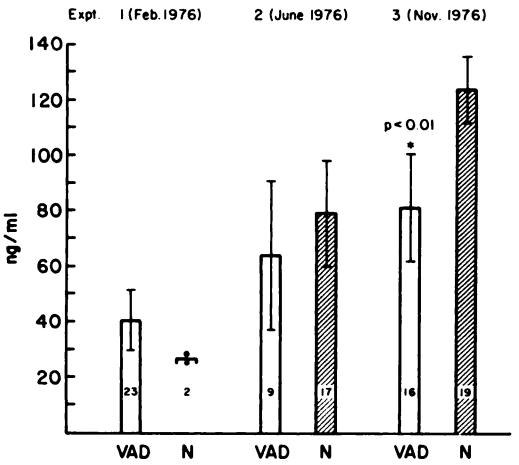

FIG. 8. Serum LH concentration of 3 groups of vitamin A deficient rats (VAD), 130-140 days of age, and age-matched normal rats (N), expressed as mean ± 2 SEM. Number inside each bar represents the number of animals used in each group. The dates assays were performed for each experiment are indicated above each pair of bars. Each pair of bars represents a single experiment with its own control group. * Indicates that the difference between VAD rats and normal rats is significant at the P value indicated above the bar.

chromatin pattern is similar to that of pachytene nuclei (Fig. 4A). However, the short time interval following [^{3}H]-thymidine injection and the absence of orderly meiosis make it more likely that these represent degenerating meiotic nuclei. Thus, these results demonstrate that the loss of germ cells in VAD rats is due to the specific requirement of vitamin A, or retinol, for normal meiosis beyond the preleptotene stage. This dependency upon vitamin A is supported by the rapid resumption of meiosis and the appearance of pachytene spermatocytes within 10 days following vitamin A treatment. The mechanism of retinol action upon meiotic prophase is not clear and deserves further investigation.

Elevation of serum FSH in VAD rats at mature age (130 days) was not unexpected in

TABLE 1. Serum LH, FSH and testosterone concentration of VAD, PVA-VAD and normal male rats.

Group	LH[ab]	FSH[ab]	Testosterone[c]
VAD	60 ± 20 (9)	766 ± 127 (9)*	1.16
Days PVA			
10	141 ± 78 (5)*†	967 ± 189 (5)*†	1.16
20	193 ± 8 (5)*†	1023 ± 549 (5)*†	0.81
30	117 ± 28 (4)*†	897 ± 74 (4)*†	0.75
40	151 ± 54 (6)*†	1041 ± 284 (6)*†	0.19
50	136 ± 25 (6)*†	858 ± 139 (6)*†	0.52
60	108 ± 26 (6)*†	543 ± 100 (6)	...
Normal control	79 ± 19 (9)	493 ± 127 (9)	2.53

[a]Mean ± 2 SEM ng/ml.

[b]Numbers in parentheses represent the number of animals used.

[c]Values for testosterone represent mean of duplicate measurement of equal volume of pooled sera from 4-10 rats. *Significantly different from normal rats ($P < 0.01$); † Significantly different from VAD rats ($P < 0.01$).

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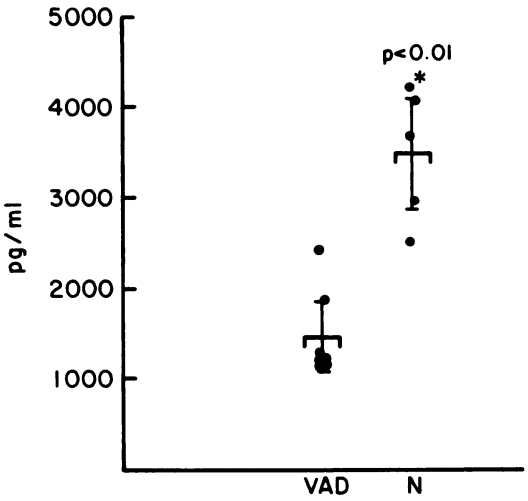

FIG. 9. Serum testosterone concentration of vitamin A deficient rats (VAD) and age-matched normal males (N), expressed as mean ± 2 SEM. Each point represents the measurement of pooled sera from 4-10 rats from all 3 experimental groups. *Indicates that the difference between the 2 groups is significant at the P value indicated above the bar.

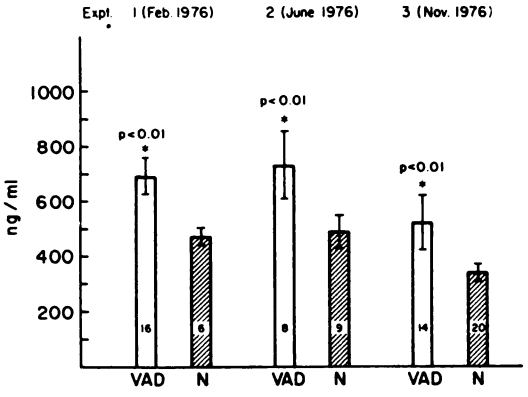

FIG. 10. Serum follicle stimulating hormone concentration of 3 groups of vitamin A deficient rats (VAD) 130-140 days of age and agematched normal rats (N), expressed as mean ± 2 SEM. Number inside each bar represents the number of animals used in each group. *Indicates that the difference between VAD rats and normal rats is significant at the P value indicated above the bar.

view of the severe depletion of the germinal epithelium. Elevation of FSH has also been reported in VAD rats at older age (160-200 days) (Rich and de Kretser, 1977), in rats whose germinal epithelium is depleted by x-irradiation (Bain and Keene, 1975; Verjans and Eik-Nes, 1976; Hopkinson et al., 1978) hydroxyurea feeding (Rich and de Kretser, 1977) alkylating agents (Gomes et al., 1973; Debeljuk et al., 1973) and in patients with azoospermia and severe oligozoospermia (Franchimont, et al., 1972; de Kretser et al., 1974). However, the FSH results herein are in contrast to the normal levels reported by Krueger et al. (1974) in VAD rats and by Mecklenburg et al. (1975) in hydroxyurea fed rats. Absolute FSH values measured in the present study and that of Krueger et al. (1974) were similar and the range of normal FSH values was the same. In addition, the age of the

TABLE 2. Reproductive performance and epididymal sperm count of PVA-VAD rats.

	Group					
	A 60 days PVA[a]	B 80 days PVA	C 100 days PVA	D 150 days PVA	E 3 μg VA rats[b]	F Normal rats
n tested	10	...	7	5	3	7
n fertile	0	...	3	3	2	7
Litter size	0	...	7.3	12	11	11
Epididymal sperm count (X 10^6)	[5][c]	3.70(8)[d] [6]	126.8(3) [7]	180.09(4) [9]	385.30(4) [7]	504.88(5) [4] 359.00 (8) 10 wks

[a]PVA = Post-vitamin A feeding.

[b]Given 3 μg vitamin A daily instead of retinoic acid.

[c]Numbers in brackets show approximate age in months at sacrifice.

[d]Numbers in parentheses represent the number of animals used.

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VAD rats when studied was the same in both observations. Thus, the discrepancy between the FSH data in this study and that of Krueger et al. (1974) cannot be explained by age or assay differences.

Because the LH levels in VAD rats studied at 130 days were normal to low, in the presence of low testosterone levels, it is possible that vitamin A deficiency may also be associated with a partial defect in LH secretion. Studies of Rich and de Kretser (1977) found that the low testosterone levels in VAD rats and hydroxyurea treated rats were associated with elevated LH values, suggesting some degree of compensated Leydig cell failure. In the present study, the observation that serum testosterone levels failed to respond to the elevated serum LH after vitamin A treatment also suggests that vitamin A deficiency causes hyporesponsiveness of the Leydig cells to gonadotropins. Therefore, the differences between the results observed in various studies of VAD rats may be a reflection of differential effects upon more than one component of the hypothalamic-pituitary-testicular axis, of differences in the preparation of the vitamin A deficient animals and in the duration of the vitamin A deficiency.

The reason for the variability in the absolute LH levels observed among different experiments in this study is not apparent. The small number of normal animals in experiment 1 and the different sources of blood (heart puncture in experiment 1 vs bleeding from the retro-orbital sinus in experiments 2 and 3) may explain the differences found. However, the significance of the low normal values is corroborated by similar decreases in serum testosterone, a result in agreement with other studies of VAD rats (Mason, 1933, 1939; Mayer and Truant, 1949; Krueger et al., 1974; Rich and de Kretser, 1977).

After vitamin A feeding, both FSH and LH levels were significantly increased within 10 days. This elevation may reflect an increased sensitivity of the pituitary to GnRH, or an increase in the sensitivity of CNS to low circulating androgen, or to the absence of inhibin (McCullagh, 1932), or the Sertoli cell factor (Steinberger and Steinberger, 1976). The return of FSH to normal values by Day 60 PVA provides evidence that completion of spermatogenesis is important in the feedback regulation of serum FSH.

Rich and de Kretser (1977) suggested that reduced ABP production in VAD rats reflected impaired Sertoli cell secretory function. The initial increase in seminiferous tubule diameter following vitamin A feeding coincides with active spermatogonial proliferation (Huang and Hembree, 1978) and a further elevation of FSH PVA (Huang et al., 1978). This finding suggests that the initial enlargement of the seminiferous tubules may reflect an action of vitamin A upon the Sertoli cell and, concomitantly, upon its secretory ability. The gradual increase in tubular diameter probably occurs to accommodate the expanding spermatogenic population.

Reinitiation of spermatogenesis occurred immediately following vitamin A feeding in the presence of low serum testosterone. This confirms that the partial testosterone deficiency was not responsible for the lack of germ cell development in VAD rats and suggests that the elevated FSH was a reflection of the severe germ cell depletion. In spite of persistently low circulating testosterone, completion of spermatogenesis and eventual return of LH and FSH levels toward normal occurred within 60 days, slightly greater than the expected duration of spermatogenesis. The timing of the sequential appearance of spermatocytes, spermatids and spermatozoa in the seminiferous tubules suggests that the initial response of germ cells is kinetically normal. On the other hand, the gradual increase in a) the percentage of seminiferous tubules containing PAS positive granules, b) the epididymal sperm count and c) the litter size in fertile rats demonstrates that the regeneration of numerically normal spermatogenesis in PVA-VAD rats depends on the repopulation of the germinal epithelium from early spermatogonia and requires more than 5 months. Undoubtedly, stem cell renewal, with repopulation of the spermatogonial compartment, must be a major determinant of the extent to which spermatogenesis becomes quantitatively normal in this model system. However, the capability of siring live offspring provides direct evidence of physiologically and, therefore, biochemically normal spermatogenesis in PVA-VAD rats.

In the present study, the temporal relationship of spermatogenic regeneration, the histologic appearance of the germ cells, the sperm production and the fertility of animals support our hypothesis that spermatogenesis reinitiated by vitamin A feeding of VAD rats is qualitatively normal. In the VAD rat, mitotic division of spermatogonia occurs in a well-defined

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endocrine environment; complete meiosis and spermiogenesis are absent. The rapid response of germ cells to vitamin A provides a unique opportunity to study the role of vitamin A in the physiology of spermatogonial development and upon the initiation of meiosis in an in vivo system. Since the timing of the appearance of later cell types after vitamin A treatment can be accurately predicted, the PVA-VAD rat may also provide an important animal model for the study of the biochemical characteristics of each stage of germ cell development.

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