THE SPERMATOGENIC CYCLE OF *MICROTUS OCHROGASTER* AS DETERMINED BY AUTORADIOGRAPHIC TECHNIQUES

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION AND LITERATURE REVIEW

The reproductive physiology of Microtus ochrogaster has been studied (Gier and Cooksey, 1967; Janes, 1962; Shepherd, 1970) with major emphasis on the female. While some work (Gier and Cooksey, 1967; Janes, 1962) has focused on the male system, no information exists concerning the timing of spermatogenesis. As M. ochrogaster is coming into wide use as a laboratory animal, the need for establishing reproductive patterns is apparent.

Early cytological evaluations of the cells comprising the seminiferous epithelium in other species have demonstrated the occurrence of cellular associations (Brown, 1885; Curtis, 1918). The series of changes occurring in a given area of the seminiferous epithelium between two successive appearances of the same cellular association, or stage, is a "cycle" of the seminiferous epithelium (Leblond and Clermont, 1952). Clermont (1960) described 12 stages of a cycle in the guinea pig recognizeable by cytological differences in maturing spermatids which were stained by the PAS technique.

The duration of each of 12 stages of a cycle of the seminiferous epithelium in the mouse was delineated by Oakberg (1956b) by selectively destroying type B spermatogonia with x-rays. As a result of this destruction, stage 7 of the cycle gradually lost its population of resting primary spermatocytes. The loss was twofold. First, no new resting primary spermatocytes arose as their parent cells (type B spermatogonia) were destroyed and secondly, those resting primary spermatocytes which were present were lost by the maturation process. During the next cycle the pachytene primary spermatocytes of stage 7 were lost in the same fashion. This same chain of events eventually led to the loss of maturing spermatozoans upon completion of the third cycle. Time involved for progressive disappearance of cell generations,

provided a basis for determination of the duration of the stages.

Autoradiographs of tissues incorporating tritiated thymidine (^{3}H-dThd) have been used to determine the rate of cellular proliferation in the mouse as "classical histological techniques do not allow for adequate study of dynamic processes, and autoradiography is proving increasingly valuable for following these processes at the cellular level" (Hughes et al., 1958).

The rationale for the use of ^{3}H-dThd is that it is exclusively incorporated into DNA and remains with the labeled cells or their descendents until death (Baserga and Malamud, 1969; Gude, 1968). Beta particles emitted from tritium have a maximum energy of .0186 Mev (Brucer, 1968) and an average range in tissue of about 1.5 μ permitting autoradiography of high resolution (Lajtha and Oliver, 1959). ^{3}H-dThd is an almost instantaneous label when used in vivo, being either incorporated or catabolized within 30 to 40 min to non-utilizable products (Baserga and Malamud, 1969). Odartchenko and Pavillard (1970), however, were able to detect free ^{3}H-dThd in testis tissue following intraparenchymal injection and believe that chromosome labeling was continuous for at least two days because of continued presence of the free ^{3}H-dThd. According to Feinendegen (1967) one DNA catabolite, thymidine or its nucleotide, is released from damaged or dying cells for recirculation and re-incorporation into DNA. Reincorporation in these circumstances is autoradiographically detectable, but it is not a serious problem unless extensive necrosis of labeled cells has occurred (Baserga and Malamud, 1969).

The toxicity of tritium has far-reaching implications and complicates study of natural cell dynamics. Artifacts introduced by the use of ^{3}H-dThd are an ever present danger as pointed out by Kisieleski et al., (1964) who found a significant reduction of resting primary spermatocytes per tubule

following ip injection of 10 μc/g body weight. Johnson and Cronkite (1959) reported 7% mortality of resting and early leptotene primary spermatocytes following ip injection of 1.0 μc/g body weight.

Artifacts may also be introduced by unlabeled dThd as experimental addition of excess quantities to cells in culture stops DNA synthesis. Apparently the four bases required for DNA biosynthesis must be present in near equal amounts for DNA synthesis to occur (Rubini and Matsui, 1968).

^{3}H-dThd is especially useful in determining the time involved for sperm to mature as it is incorporated only into DNA of spermatogonia and "resting" primary spermatocytes as no further DNA synthesis takes place in the more highly developed cell types (Monesi, 1962). The time required for the appearance of label in cell types more highly developed than "resting" primary spermatocytes, is then equal to the time required to develop to that type from "resting" primary spermatocytes. While mitochondrial DNA has been shown to incorporate ^{3}H-dThd (DeRobertis et al., 1970), and repair synthesis may occur after damage to DNA, uptake of ^{3}H-dThd under these circumstances is not demonstrable with current autoradiographic techniques (Rubini and Matsui, 1968).

Activities in the seminiferous epithelium of the boar were divided by Swierstra (1968) into eight stages based on the morphology and cytology of the germ cells and their relative positions within the seminiferous tubule. Tissue samples for autoradiography were taken from animals at various times following ear vein injection of ^{3}H-dThd and the duration of a cycle was determined to be 8.6 $\pm$ 0.1 days. Newly formed spermatids and mature sperm were found labeled at 11.5 and 25 days post-injection respectively.

Samuels and Kisieleski (1963) compared the uptake of ^{3}H-dThd by the testes

with the uptake by other organs in the mouse following ip injection, but the variation between retained activity in the left and right testes or a comparison of anterior to posterior portions of the same testis has not been investigated. Numerous factors influence the incorporation of exogenous precursors into DNA (Feinendegen, 1967; Swenerton et al., 1969; Toliver and Consigli, 1971), among them the routes of administration (Feinendegen, 1967).

The scope of this work was primarily intended to indicate the time spent by a cell, as a particular type, during the maturation process, i.e., lepotene, zygotene, pachytene, diplotene, etc., and secondarily to explore the variation in uptake of ^{3}H-dThd by the testes as affected by method of application and portion of the testis involved.

MATERIALS AND METHODS

Animals used in these studies were sexually mature, virgin M. ochrogaster males, from a colony maintained at KSU (Gier and Cooksey, 1967). Animals were raised in individual cages from weaning and except where indicated received food and water at libitum.

Study I

To determine the point at which the testicular artery could be most easily injected with ^{3}H-dThd thus allowing for availability of the total quantity to the testis, the route by which the blood entered the testis was studied in four groups of five animals each, injected with either latex (two groups), elemental mercury, or radio-opaque medium (Hypaque from Winthrop Laboratories).

Following ether anesthesia, an incision was made along the entire ventral surface of each animal, exposing all viscera. Injection was made into the left ventricle, and the heart was massaged after latex and mercury injections until the medium had progressed to the visible termination of the testicular artery. For the Hypaque injections, massage was unnecessary as the medium was serous and circulated readily. However, as the medium was colorless and miscible with blood, determination of entrance of the fluid into the testicular circulation could only be approximated, so 30 sec after injection both the aorta and post cava were severed, to halt circulation of the medium.

Both testes from each latex-injected animal in one group were excised, fixed in formalin overnight, washed, and dissected under a stereo microscope. The testes from the other latex-injected group were excised, fixed in formalin, washed in water and then placed in 1% KOH solution to digest the connective

tissue, thus leaving the latex filled vessels clearly visible when examined under a stereo microscope. All the testes from the elemental mercury, and Hypaque-injected animals were excised and x-rayed without fixation.

Study II

Animals were injected by each of three methods; tissue samples prepared, radioactivity determined, and statistical tests employed, to establish which method was the most efficient on the basis of retained activity, distribution of isotope, and time involved in the operation.

Three groups of eight animals each were fasted for 8 hours, anesthetized with sodium pentabarbital (Nembutal, Abbott Laboratories), and each group injected with 1 µl thymidine-methyl-^{3}H (sp act 6.7 c/mM) diluted to a final concentration of 0.25 µc/µl of physiological saline. This dosage was used on the basis that Hahn (1970) showed the average testis weight of sexually mature M. ochrogaster was 250 mg and Gude (1968) recommended 1 µc/g as proper for iv injections.

Immediately before injection 1 µl of the ^{3}H-dThd solution from an automatic dispensing microliter syringe (Hamilton Co.) was placed on a hydrophobic plastic sheet, drawn into a glass microinjection needle (mechanically pulled, micro-manipulation capillary tube) connected by means of plastic tubing to a gas-tight syringe (East Rutherford Syringes, Inc.) and the solution extruded at the chosen site. Needles were discarded after one use, and aseptic technique was observed during all surgical procedures. Intraperitoneal and intra-arterial injections were performed under a stereo microscope.

Treatment Group 1 (VTA): A mid-scrotal incision was made on the ventral left side through the tunica vaginalis (Gier and Marion, 1969). The injection

was made via the testicular artery on the ventral aspect of the posterior portion of the testis (fig. 1). The testis was then returned to its normal position and the incision sutured.

Treatment Group II (VIP): A mid-abdominal incision was made through the parietal peritoneum and the peritoneal surface reflected. The isotope solution was placed on the peritoneum and the incision closed.

Treatment Group III (VIT): A ventral mid-scrotal incision was made on the left side to the tunica vaginalis. Injection was accomplished after passing the needle through the tunica vaginalis and tunica albuginea into the parenchyma of the posterior portion of the testis. The incision was then sutured.

Two individuals from each treatment group were sacrificed by ether anesthesia at 0.25, 1, 24, and 48 hours post-injection. Both testes from each animal were excised and a length of 35 gauge wire inserted through the posterior portion of each as markers. They were then wrapped in aluminum foil and frozen in liquid nitrogen.

While frozen, each testis was cut into approximately equal anterior and posterior portions. Each portion was weighed to 0.1 mg, placed in a Potter-Elvehjem homogenizer tube with four volumes of water, and homogenized. After homogenization, a 0.15 ml aliquot of homogenate was transferred to a scintillation vial and 0.75 ml of NCS tissue solubilizer (Amersham/Searle Corp.) added. The vials were sealed and placed in a 50° C water bath for 12 hr to insure complete digestion of the tissue.

After the digestion period, 19 ml of PPO (2,5 diphenyloxazole) - POPOP (1,4-bis [2-(5-phenyloxazolyl)]-benzene) in toulene (9.8 g PPO, 0.2 g POPOP in 2000 ml toulene) were added to each vial in accordance with procedures of

Feinendegen (1967) and Amersham/Searle Corp. (1969).

Six background samples were prepared from testes of untreated animals following the procedures just outlined and counted with the 96 other samples in a Packard 3200 scintillation spectrometer for 20 min each. External standard values were also obtained for each sample.

To correct for quenching the following procedure was used. After each sample had been counted and an external standard value obtained, three samples which represented high, medium, and low degrees of quenching, as determined from the external standard values, were chosen to be used for internal standardization. Five μl of n-hexadecane 1-2-T (sp act 2.47 μc/g)calculated to contain 18,526 dpm at the time of use were added to each of these vials and total counts for 20 min and external standard values again obtained. The initial count-rates of the three samples were subtracted from the count-rates obtained after n-hexadecane was added, and a graph constructed, plotting the percent counting efficiencies against the external standard values. The external standard values were equal in both determinations for the three samples. The percent counting efficiency was then determined for each of the other samples from the graph prepared from the internal standards.

Corrections for background and quenching were applied to each sample, as outlined by Feinendegen (1967). Values were calculated for dpm/mg of tissue, and for dpm/total testis portion, then means were determined for two animals for each sacrifice time of the three treatment groups.

Statistical analyses of the data employed both non-parametric (Wilcoxon and Wilcox, 1964) and parametric tests where appropriate. Parametric analyses used $\log_e$—transformed data and were done on KSU's IBM 360/50 computer using R. R. Kowal's double precision, FORTRAN program, GMANOV. This program tests

general linear hypotheses as described by Morrison (1967). The univariate linear model used was $X_{ijk} = u_{ij} + E_{ijk}$ where i = 1, 2, 3; j = 1, 2, 3, 4; and k = 1, 2 indexed injection routes, sacrifice times, and replicates respectively.

Study III

The cytology of the seminiferous epithelium was studied and autoradiographs prepared to determine the duration of spermatogenesis.

Testes from three animals were fixed in Bouin's fluid for 24 hr, washed overnight in water, dehydrated, infiltrated with paraffin, section at 4 μ, mounted on glass slides and stained with Harris' hematoxylin. As nuclear morphology is variable among species, the cells of the seminiferous epithelium of M. ochrogaster were compared to illustrations given for the rat (Daoust and Clermont, 1955), guinea pig (Clermont, 1960), hamster (Clermont, 1954), mouse (Oakberg, 1956), bull (Gillette and Corwin, 1964), and drake (Clermont, 1958) and a classification system constructed.

Twenty-seven animals were injected by the procedure described for treatment group I (VTA) of study II, and sacrificed by ether anesthesia at 0.25, 1, 3, 6, 12, and 18 hr, and 1, 2, 3, 4, 6, 7, 8, 9, 12, 14, 16, 18, 20, 25, 30, 35 and 40 days post-injection. The left testis of each animal was excised, fixed in Bouin's fluid for 24 hr; washed overnight, dehydrated, infiltrated with paraffin, sectioned at 4μ, and mounted on glass slides (Gude, 1968).

De-paraffinized slides were coated by the dipping technique with NTB-2 nuclear track emulsion (Eastman Kodak Co.) diluted with double distilled water (1:3 v/v), as outlined by Rogers (1967). The dipping solution was maintained at 42° C throughout the procedure. After drying for 1 hr in a

28° C, 60% relative humidity atmosphere, slides were placed in light-tight plastic boxes with 5 g of desiccant (Drierite, Hammond Co.). The boxes were wrapped in aluminum foil, placed in polyethylene bags, and maintained at 4° C until developing time.

After 12, 30, and 85 days of exposure, slides were developed in Dektol for 2 min, rinsed in distilled water for 10 sec and fixed in f-5 fixer for 5 min. All solutions were maintained at 15° C (Eastman Kodak Co., 1966). After fixing, the slides were rinsed in tap water for 10 min and stained with Harris' hematoxylin.

Slides of each testis from each of the three exposure periods were randomly selected and microscopically studied. As each slide contained three or more cross-sections of testis, one cross-section was chosen and all tubules observed. The labeled cell types considered to be the most highly developed, as determined by the nuclear characteristics, were recorded, with the exception of those slides showing labeled "maturing" spermatids and/or mature sperm, in which case all labeled cell types were noted. Cell types were considered as being labeled if the number of reaction foci overlying their nuclei was higher than background foci in that area of the tubule.

RESULTS

Study I

Dissection of the latex-injected arterial systems revealed that the paired internal spermatic arteries arise from the aorta just posterior to the renal arteries. Each artery passes posteriorly and ventrally to the testis and upon reaching the fat pad, anterior and dorsal to the testis, bifurcates giving rise to the epididymal and testicular arteries (figure 2). The testicular artery becomes highly tortuous prior to reaching the testis, and except for the color of the injection medium would be indistinguishable from the mass of venous vessels which surround it at this point. This association of arterial and venous vessels is referred to as the pampiniform plexus. Upon reaching the testis proper the testicular artery passes through the tunica albuginea, posteriorly along the dorsal aspect, around the curvature of the inferior pole, and then anteriorly along the ventral aspect. Until reaching the ventral aspect of the testis, the artery is rectilinear but on the ventral surface it begins to meander; between the equator and the superior pole it disappears into the parenchyma at a shallow or steep angle, depending on individual variation. The artery penetrates to the center of the testis where it divides into two large branches which in turn give rise to smaller vessels. Each of these larger branches seemingly give rise to smaller vessels which supply the anterior portion of the organ. No collateral branches of the testicular artery were found to enter the parenchyma at a point other than that described. In one case, however both testicular arteries of an individual bifurcated after reaching the ventral aspect of their respective testes but rejoined prior to entering the parenchyma.

The injected testes that were digested with KOH gave a more refined picture of the arterial system and for the most part were in accord with observations on the dissected specimens. In addition, the 10 testes prepared in this fashion showed small collateral branches which supplied the anterior portion, emanating from the main trunk after it entered the parenchyma, but before the division into two major trunks. In one case, both testicular arteries of an individual gave off two collateral branches on the ventral aspect of the inferior pole. These however were evident even before latex injection. A few mm from the point of origin each vessel disappeared into the parenchyma. After injection and digestion these arteries were traced from their origins to the capillary beds they supplied. In all four cases these vessels were less than .05 mm in diameter at their origins in contrast to 0.25 mm diameters of the main trunks from which they arose.

Radiographs of testes injected with elemental mercury clearly defined the larger vessels. Smaller vessels, however, were for one reason or another not filled, and therefore no conclusion concerning the existence of collateral branches could be reached.

Radiographs made from Hypaque-injected testes had rather poor resolution and as the injection medium was distributed in venous as well as arterial portions of the vasculature it was extremely difficult to determine whether some vessels were collateral branches of the testicular artery or were veins crossing the artery, which on a two dimensional plate may appear to be collateral branches.

In summary then, the testicular artery, except in two anomalous individuals, is a single vessel having no collateral branches between its point of origin and the point it enters the parenchyma of the testis.

Study II

Weights of testes portions, retained activities per mg, and total retained activities are given in table 1. Mean values for these measurements are given in tables 2, 3, and 4 respectively.

The Wilcoxon signed rank test showed that no significant weight differences existed within treatment groups among: anterior vs posterior testes portions; right anterior vs right posterior; or left anterior vs left posterior.

For retained activities (dpm) among testes portions within treatment groups the Wilcoxon signed rank test showed significant differences (two-tailed) in the left anterior vs left posterior in VTA treatment ($P = 1.0\%$); right anterior vs right posterior in VIP treatment ($P < 5.0\%$); left anterior vs left posterior in VIT treatment ($P = 1.0\%$). Those tests in which no significant differences occurred are: right anterior vs right posterior in VTA treatment, left anterior vs left posterior in VIP treatment; and right anterior vs right posterior in VIT treatment. The eight individual left anterior portions from the VTA treatment always retained more activity than did the left posterior portions. Conversely the eight individual left posterior portions always retained more activity than the left anterior portions within the VIT treatment.

The Wilcoxon rank sum test (two-tailed) showed that the left testes from the VIT treatment had a significantly greater difference in retained activity between the anterior and posterior portions than the anterior and posterior portions of the VTA treatment ($P < 1.0\%$). Stated another way, the retained activity was more evenly distributed between the anterior and posterior portions of the testes in the VTA treatment than in the VIT treatment.

Analysis of variance showed significant differences (table 5) in retained activities, in the left testes among the three treatment groups. Anterior and posterior portions of the left testes similarly showed significant differences, whereas the right testes, or the anterior and posterior portions of the right testes did not show significant differences in retained activities.

Between group comparisons of retained activities in similar testes and portions, showed no significant differences when the right testes or portions were considered (table 6). The left testes and portions of the VTA and VIT treatment groups however showed significant differences when compared to the VIP treatment; the VTA and VIT treatments retained more activity. The left testes of the VTA treatment group showed no significant difference in retained activity when compared to the left testes of the VIT treatment, though significant differences existed between both the anterior portions and the posterior portions of the left testes of the two treatments.

While tables 3 and 4 show trends in fluctuation with regard to retained activities vs time from injection to sacrifice, comparisons of retained activities (table 7) in similar testes within treatments, as a function of time, were not significant.

Although the time required for injection of animals in the VTA, VIT, and VIP treatment groups were 15, 11, and 3 min respectively, the VTA method was considered to be the most desireable because of more even distribution than the VIT treatment and higher retention than the VIP treatment.

Study III

The cells of the seminiferous epithelium of M. ochrogaster most closely resembled those described by Gillette and Corwin (1964) for the bull. The classification of cells in M. ochrogaster along with those of other species that most closely resembled them are given in table 8, and the various cells of the classification system used for M. ochrogaster are illustrated in figures 5 to 13, and 18.

Autoradiographs revealed that 0.25 hr post-injection leptotene primary spermatocytes had become labeled, indicating that the preleptotene or resting primary spermatocyte undergoes rapid transformation once the synthetic period is over. No cells later than leptotene primary spermatocytes were labeled at 0.25 hr post-injection.

Nine days post-injection the last stage of meiotic prophase was labeled, and labeled "round" spermatids were first detected 10 days post-injection (table 9). At 16 days post-injection all 3 types of spermatids were labeled, while at 18 days only the two higher developed types, "maturing" and "elongate" spermatids were observed along with mature sperm free in the lumina. By 20 days the only remaining labeled cells were terminal stages of maturing spermatids and mature sperm free in the lumina. No label was found in any cell types 25 to 40 days post-injection.

Although autoradiographs from each of the three exposure periods were studied, only those exposed for 85 days contained reaction foci. The labeled cell types encountered at the various intervals after injection are listed in table 9, and illustrated in figures 16 to 19.

DISCUSSION

The arterial circulation of the testes in M. ochrogaster was in close agreement with the description given by Janes (1962), but more precise knowledge was needed as Janes' work did not illustrate the proximity of arteries to veins. This relationship was of primary importance as accidental iv injection of isotope would yield misleading results with regard to the percentage of isotope retained by the testes.

First-hand information regarding the most suitable site for injection was also needed as any difficulty in manipulation of the artery during the injection procedure might also produce misleading results. Oettle and Harrison (1952) pointed out that in the rat acute temporary ischemia of 10 to 20 min duration produced hyperchromasia of spermatogonia, which was detectable for 2 weeks. Techniques employed for arterial injection of M. ochrogaster impeded the flow of blood for a maximum of 15 sec as a result of arterial constriction following the removal of the micro-injection needle, but was not considered to be of any consequence in the alteration of the spermatogenic process. The point at which injection was made was the most accessable and was virtually unmistakeable for a vein. As the artery was held in place by the underlying tubules and the overlying tunica albuginea it was easily injected without undue manipulation.

The use of radio-isotopes to study spermatogenesis in relatively small laboratory animals can be difficult. The size of the animal greatly limits the techniques available for the administration of the tracer, and perhaps accounts for the common practice of injecting the isotope intraperitoneally. The mean retention of ^{3}H-dThd by the testes within the VIP treatment group was 2,820 dpm, or approximately 0.5% of the total dose administered (table 4),

which is comparable to results calculated from the work of Kisieleski et al. (1964). Although they did not state the weights of the mice they injected with ^{3}H-dThd (0.1 μc/g of body weight) they stated the age as 14 days. As 5 g is a good approximation of body weight for this age then the retention by the testes was approximately 0.8% of the total dose administered and is in close agreement with our findings even though the total body weights were considerably different.

These results indicate that ip injection of ^{3}H-dThd is unspecific for testis tissue and even if autoradiographically detectable amounts are present in the testes the procedure is uneconomical because the major portion of the injection is wasted. Johnson and Cronkite (1959) reported that "excellent autoradiograms for the study of cell turnover can be obtained after doses of only 0.05 to 0.5 μC/gm", but failed to report the type of animal used in those studies or list direct references to those studies. As in the same paper, 4 weeks were required to produce worthwhile autoradiographs after ip injection of 20.0 μc/g of body weight, it seemed illogical to use less than 1/40th this quantity. It was also pointed out by them that a dose of 0.5 μc/g of body weight, injected ip into mice, resulted in a 3% mortality of spermatogonia. These results were of limited value because only the total amount injected was stated so only this quantity can be equated with cell death regardless of the fact that uptake by the testes may be variable among individuals with the same body weight. While this variation may not exist in other experimental animals, unpublished data (Gier) indicates that body weight alone is a poor index of testis size in M. ochrogaster. Of greater importance than the mortality resulting after a given total dose, is the cell mortality associated with the amount of retained activity in the organ under study, as it is the affinity of the target organ for the compound that will probably determine the direct harmful

effects of the isotope. Interest in this type of experimentation also needs to be focused on the retained activities in tissues other than the target organ. If less than 1.0% of the tracer is retained by the testes following ip injection, a decrease in survival of cells may be due indirectly to damage caused elsewhere in the body by the other 99%, as after ip injection of 1.0 μc/g of body weight in mice (sp act 0.36 c/mM) Samuels and Kisieleski (1963) showed that retained activity was greater in the spleen, liver, and kidneys than in the testes.

For economic reasons, and the lack of precise information in the literature regarding damage caused by tracer in other tissues of the body which may indirectly affect spermatogenesis, it seemed desireable to use a method other than ip injection, which would (1) allow for a level of retention by the testes that was not deleterious, and (2) at the same time keep the retained activity in other parts of the body to a minimum.

Retention of tracer averaged 608 dpm/mg for the testes in the VTA treatment group (table 3) and no individual testis portion showed more than 859 dpm/mg (table 1). Several individual testes portions of the VIT treatment group as a result of poor distribution, showed more than 2000 dpm/mg (table 1), and as this level was above the 1440 dpm/mg which Kisieleski et al. (1964) found to be harmful to resting primary spermatocytes only the intra-arterial injection method filled both of the qualifications set up. Odartchenko and Pavillard (1970) used intraparenchymal injections to study DNA replication in meiotic chromosomes of mice but did not make any reference to the possibility of damage by that method.

Results from intra-arterial and intraparenchymal injections indirectly show that a high percentage of isotope injected ip is never available for

distribution via the circulatory system.

As the left testes probably received near equal amounts of isotope from the general circulation as did the right testes in the VTA and VIT treatment group, then the mean amount retained by the left testes (table 4) minus the mean amount retained by the right testes is equal to the mean amount retained by the left testes immediately upon injection, and this quantity was never available for circulation and retention elsewhere. This quantity is the "mean immediate retained activity", and for the left testes of the VTA treatment group was 96,190 dpm or 17.3% of the total dose injected. The mean immediate retained activity in the left testes of the VIT treatment group was 156,620 dpm or 28.2% of the total dose injected. The amount which was not immediately retained is then assumed to have been taken into the general circulation for distribution to all tissue in the body. Of this amount in the circulatory system approximately 0.75% was removed by both testes (right and left) of the VTA group, and 0.74% was retained by both testes of the VIT group.

In the VIP treatment group 2,820 dpm were retained by both testes which is approximately 0.51% of the total dose injected. If we assume that this quantity (2,820 dpm) is 0.74% of that carried by the circulatory system, the total amount carried was 380,600 dpm which is 68.6% of the total dose injected; then 31.4% was never available for distribution in the body via circulation, thus limiting the amount available for the testes to retain. This however must be confirmed by other methods.

Table 3 shows that the mean retained activities/mg, in both the right and left testes among the three treatment groups is highest 1 hr post-injection. The level of retained activity then decreases, and by 48 hr post-injection is always lower than the 15 min retention level.

These same trends are evident in table 4 but analyses of the data shows that the differences in retained activities among the times are not significant. It would be expected that the amount of retained activity in the right testis from the intra-arterial and intraparenchymal injections would increase until the time at which ^{3}H-dThd was no longer available from circulation for incorporation into DNA. This increase also should occur in both testes of the VIP treatment group animals but it also was not found to be significant.

Mice labeled with three daily ip injections of ^{3}H-dThd received unlabeled skin grafts 2 days post-injection. Seven days after receiving the grafts a few epidermal cells of the grafts appeared labeled. "This means that ^{3}H-label is available for DNA-synthesis for a much longer time after labeled thymidine injection than previously assumed" (Diderholm et al., 1962). This opinion is supported by the work of Odartchenko and Pavillard (1970) who found labeling to be "continuous for at least 2 days" following intraparenchymal injection of ^{3}H-dThd into mouse testes, "as evidenced by an evaluation of free ^{3}H-dThd in testis tissue at various time intervals after injection". Staroscik et al. (1964) indicated that 95% of the uptake of this compound occurs within 25 min after injection, and as our work shows no significant differences in retention after 15 min it is assumed that the major portion is incorporated by 15 min and any further incorporation is small by comparison.

Retention of tracer, in all individuals of the VTA treatment group, was highest in the anterior portion of the injected testes (table 1). This difference was highest in animal 26 in which the anterior portion retained 5 times more activity than the posterior portion, and lowest in animal 20 in which the anterior retained only 20% more activity than the posterior. From tables 2 and 4 it was computed that the anterior portions on an average retained

twice as much isotope as did the posterior portions.

Variations which occur within the vascular network of the two portions have been considered but do not offer any indication why the difference in retention might occur. DNA synthesis could possibly cause more to be retained in the anterior portion if the rate was higher in that portion, however, there are no indications that this is the case, as the retained activities from ip injections do not show this same differential.

The time required from the incorporation of ^{3}H-dThd into DNA of stem spermatogonia until label is autoradiographically detectable in mature sperm in the lumina of the seminiferous tubules can be used as an index for the duration of spermatogenesis. Courot et al. (1970) indicated that this is actually "amputated spermatogenesis" as the stem spermatogonia have already been in existance before the synthetic (S-phase) period in which the ^{3}H-dThd was incorporated. In the mouse, calculations based on the work of Monesi (1962) and Courot et al. (1970) show that approximately 4 days are spent by the stem cells in the G_1-phase or presynthetic period.

In M. ochrogaster only the latest labeled cell types were noted up to the time at which maturing spermatids became labeled because it is only the latest cell type which can be shown to be the direct descendent of the resting or preleptotene cell initially incorporating the tracer. Cell types which are labeled and are not the latest labeled cell types present are either the descendents of cells which became labeled during the S-phase of spermatogonia or during the early stages of the S-phase of preleptotene spermatocytes.

Courot et al. (1971) stated that in the bull and ram preleptotene and leptotene primary spermatocytes incorporate ^{3}H-dThd. To substantiate this statement they refer to a photomicrograph of an autoradiograph, of bull

testis removed from the animal 30 min following the injection of ^{3}H-dThd. No leptotene primary spermatocytes are labeled in this illustration, but in fact the latest labeled cell type according to the explanation of the plate is preleptotene. Also in direct conflict to this statement is a table that they included from Esnault (1965) which showed the DNA content of bull leptotene primary spermatocytes to be two times higher than preleptotene primary spermatocytes.

In the mouse (Monesi, 1962) the latest synthesis of DNA takes place in resting or preleptotene primary spermatocytes. Data from M. ochrogaster shows that the DNA synthesis may occur in the leptotene cell as this was the latest labeled cell type detected at 15 min post-injection, but as there is no conclusive evidence in the literature that incorporation occurs in cells later than preleptotene, but substantiating evidence that it occurs latest in the preleptotene cell, we assume that the label in the leptotene cells in M. ochrogaster is the advancing label front of the isotope which was incorporated in the preleptotene cell.

The meiotic prophase which Leblond et al. (1963) considered as being leptotene through diakinesis is quite variable among species. In man it is 23.2 days and in the boar 12.4 days. Breed variations also exist (Courot et al., 1970) as in the Sherman rat it is 12.0 days, in the Sprague-Dawley 12.9 days, and in the Wistar 13.3 days. Oakberg (1956b) showed that in F_1 mice resulting from the cross of inbred 101 females with inbred C3H males, the prophase lasted 12.7 days, while Odartchenko and Pavillard (1970) showed that in A-Swiss albino mice the prophase lasted 9 days.

In M. ochrogaster the length of the prophase is approximately 9 to 10 days, as extrapolated from table 9. A more exact determination is not possible because no animals were sacrificed between 9 and 10 days post-injection. Dictyate, the

latest labeled cell type at 9 days post-injection, and round spermatids, the latest labeled cells type at 10 days indicate that the spermatocyte divisions (1st and 2nd meiotic divisions) occur within this time period.

Spermiogenesis, the development of spermatids into mature spermatozoans, in M. ochrogaster begins between 9 and 10 days post-injection and terminates between 16 and 18 days post-injection.

The maturing spermatids present at 16 days post-injection have developed into mature sperm which are leaving the testes by 18 days. The maturing spermatids present at 18 days are thought to be the descendents of spermatogonia which became labeled, and were present at 16 days post-injection as elongate spermatids.

Oakberg (1956b) reported that in the mouse, spermiogenesis lasts 13.5 days, and (Hochereau et al., 1964 from Courot) reported in the bull it lasts 19 to 20 days, illustrating that both the length of spermiogenesis and the accuracy with which it can be measured are variable.

"The measure of the duration of spermatogenesis presumes that its beginning and term are accurately known. . .Until recently, the literature has provided conflicting data on the duration of the processes. This is explained, on the one hand, by the fact that the methods differ greatly and are of unequal value and, on the other hand, because the expressions used in order to define the duration of spermatogenesis are not always identical" (Courot et al., 1970).

DeRobertis et al. (1970) reported that preleptotene corresponds to early prophase of mitosis. This analogy indirectly conflicts with the "normal" cell cycle in mitosis in which DNA synthesis takes place during interphase. "Resting cell" which by many is equated with interphase is a misnomer and it is convention such as this which greatly stifles investigations. Mitosis should not be homolo-

gized with meiosis solely because similar terms are used in describing both processes. At present there is ample confusion in the terminology of meiosis, and specific comparisons of meiosis and mitosis only compound the problems.

An investigator of spermatogenesis must be able to classify the numerous cell types comprising the seminiferous epithelium before tabulation of labeled cells is possible. Comprehensive knowledge of descriptions offered by numerous authors is therefore a prerequisite but at the same time may be misleading if strictly followed.

The classification systems for spermatogenic cells varies directly with the number of investigators. Inconsistencies in the classification systems are apparent not only among authors but also among investigations by the same author (table 8). Perhaps such discrepancies stem from the lack of any real basis for classification. In light of such inconsistencies, the morphology of the nuclei illustrated in other works was the only reference point available for this study.

It must be remembered however that resemblance of nuclei is not necessarily an indication of similar cellular dynamics. Courot et al. (1970) stressed that: "The young spermatocyte in the preleptotene stage resembles the spermatogonia from which they arise and with which they are frequently confused". The inability to differentiate between cell types strictly on the morphology of the nucleus was brought out by Leblond and Clermont (1952) in their definition of primary spermatocyte which "...goes through the well-recognized stages of meiosis; leptotene, zygotene, (transition for early pachytene), pachytene, diplotene, diakinesis, metaphase, anaphase and telophase". These "well-recognized stages" of diplotene and diakinesis, however, are both represented by the same illustration, captioned: Di; diplotene and diakinesis.

In M. ochrogaster the chromatin threads during pachytene give the nucleus

a typical crescent appearance. The rat however does not contain any nuclei which are similar in appearance (Leblond and Clermont, 1952) although the kinetics of both "pachytene" nuclei are probably the same if morphology is not considered.

The classification system used for the cell types in M. ochrogaster is only justifiable by the fact that the progression of the labeling front follows the nomenclature. That nomenclature itself infers a chronological relationship.

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The author wishes to thank his major professor, Dr. H. T. Gier, for his helpful recommendations and constructive criticism. Without his professional guidance and faith this study could not have been completed.

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DESCRIPTION OF FIGURES

Fig. 1. Ventro-medial view of latex-injected left testis and associated structures: fat pad (FP); vas deferens (VD); cauda epididymis (CE); gubernaculum (GUB); testicular vein (TV) and testicular artery (TA). X 4.25.

Fig. 2. X-ray of left testis after injection with elemental mercury: internal spermatic artery (SA), epididymal artery (EA), testicular artery (TA), and injection point (PI) used for administration of ^{3}H-dThd. X 3.1.

Fig. 3. Lateral view of latex-injected left testis after digestion in KOH showing ramifications of testicular artery (TA) and injection point (PI) used for administration of ^{3}H-dThd. X 10.

Fig. 4. Lateral view of latex-injected left testis after digestion in KOH. Testicular artery follows atypical course after penetrating the parenchyma. X 10.

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

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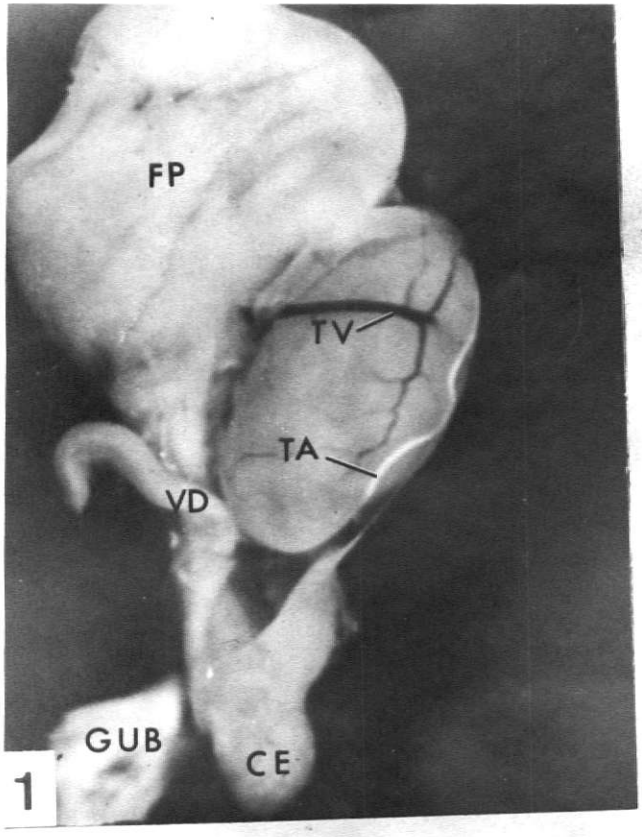

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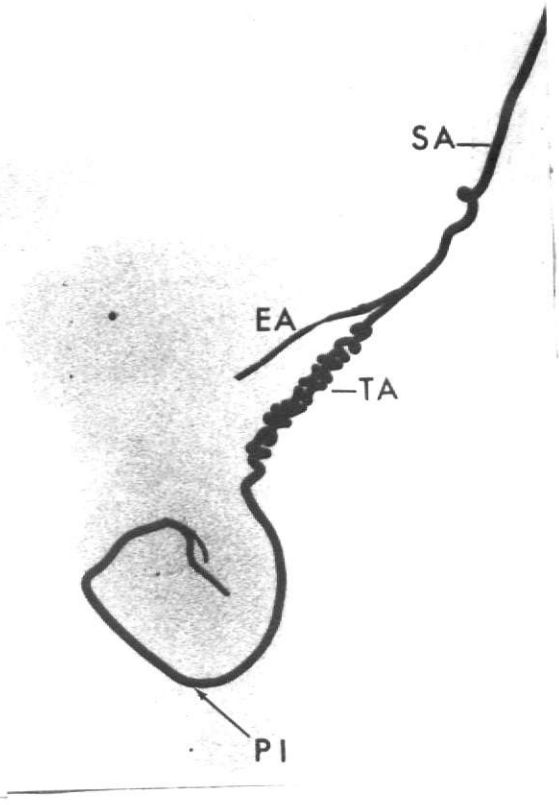

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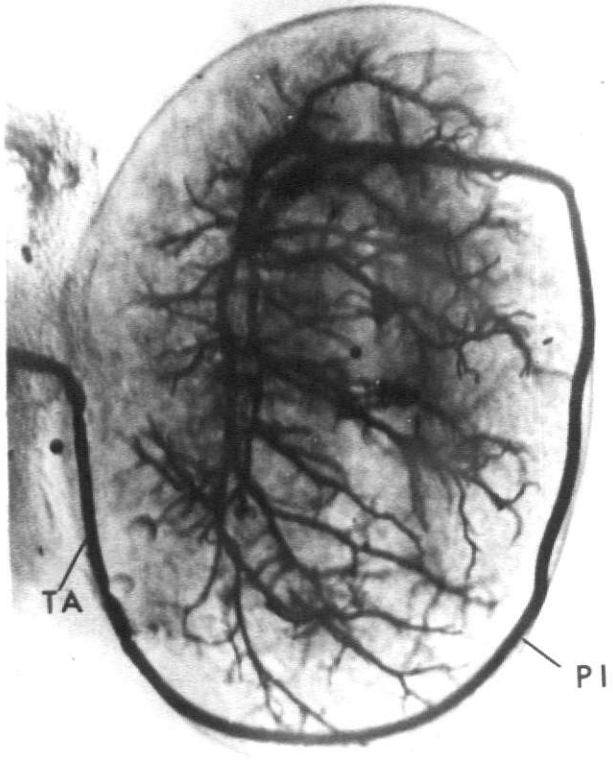

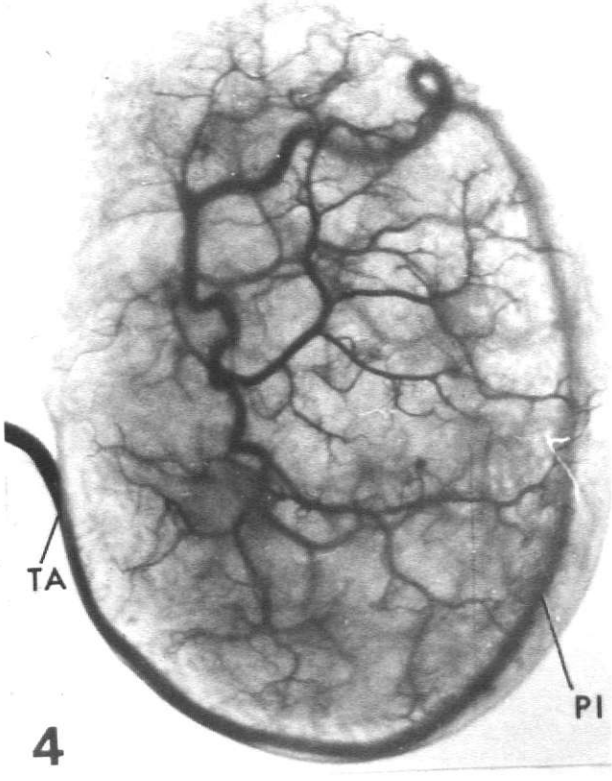

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Fig. 5. Spermatogonia A (A), diplotene primary spermatocytes and round spermatids (RS), in seminiferous tubule. X 1540.

Fig. 6. Spermatogonia B (B), diplotene primary spermatocytes, and round spermatids (RS), in seminiferous tubule. X 1540.

Fig. 7. Preleptotene primary spermatocytes (PL), diplotene primary spermatocytes (Di), and round spermatids (RS), in seminiferous tubule. Stained with iron hematoxylin-orange G. X 1540.

Fig. 8. Leptotene primary spermatocytes (L), and round spermatids (RS) in seminiferous tubule, stained with iron hematoxylin-orange G. X 1540.

Fig. 9. Zygotene primary spermatocytes (Z), and round spermatids (RS) in seminiferous tubule, stained with iron hematoxylin-orange G. X 1540.

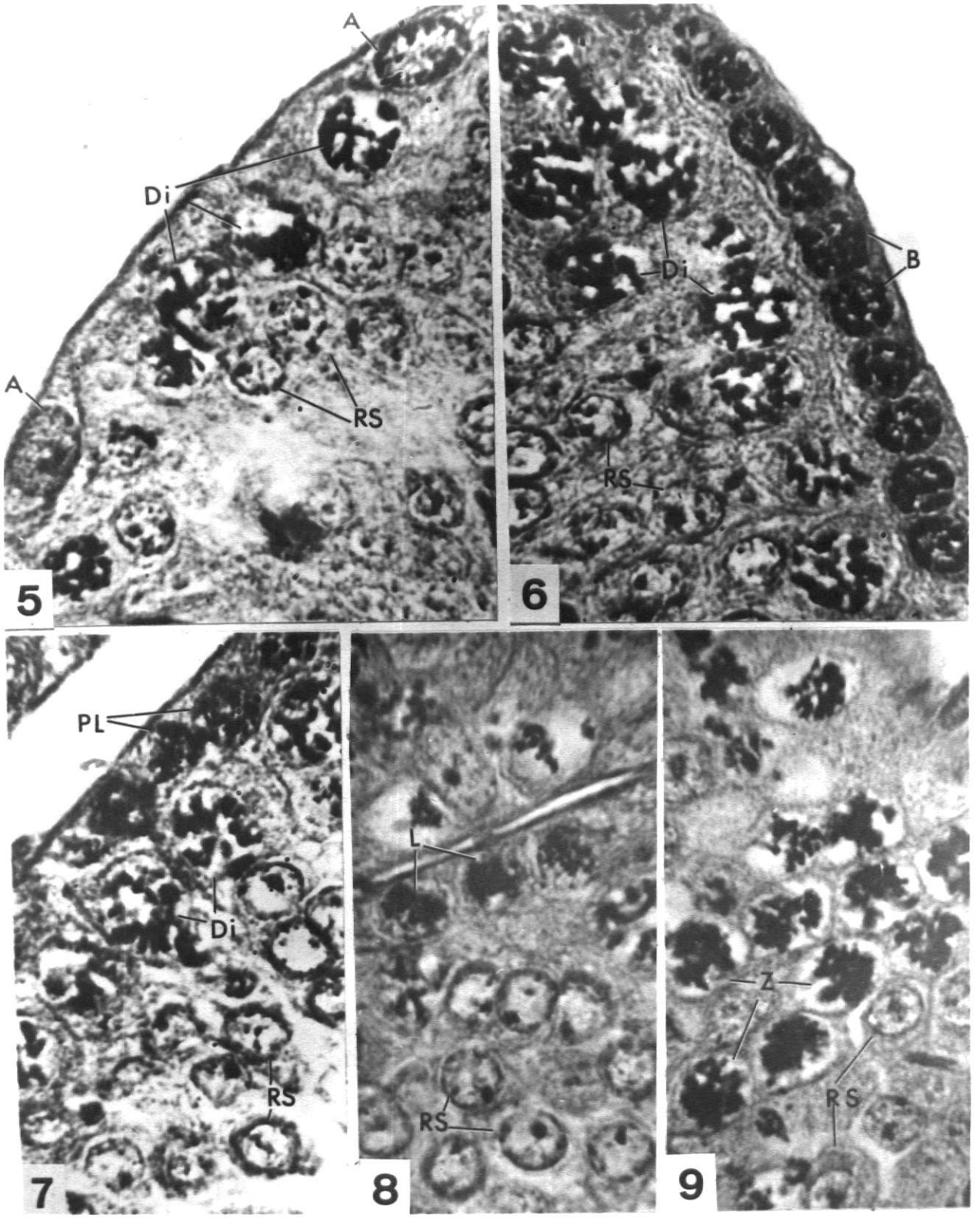
A
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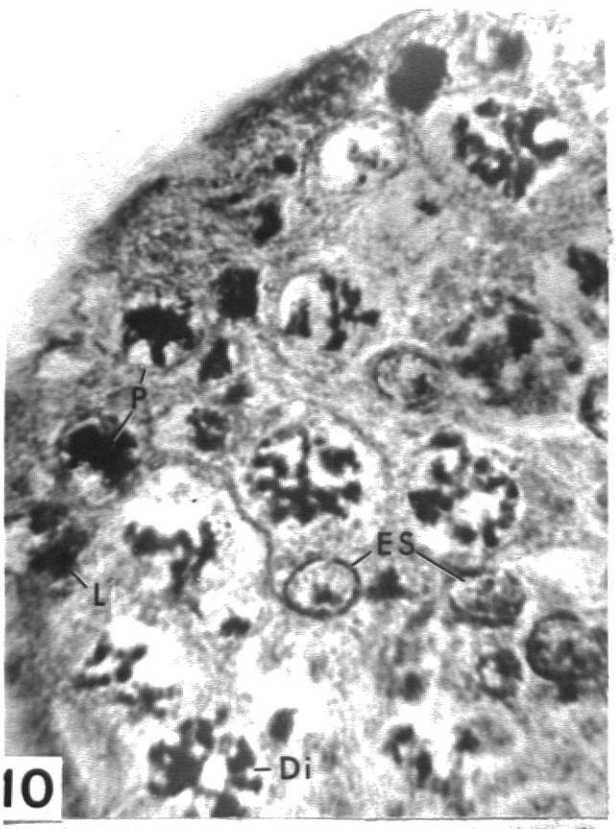
Fig. 10. Pachytene primary spermatocytes (P), leptotene primary spermatocyte (L), diplotene primary spermatocyte (Di), and elongate spermatids (ES), in seminiferous tubules. Stained with iron hematoxylin-orange G. X 1540.

Fig. 11. Dictyate or diakinesis of primary spermatocytes (DK), and elongate spermatids (ES), in seminiferous tubule. Stained with iron hematoxylin-orange G. X 1540.

Fig. 12. Elongate spermatids (ES), diplotene primary spermatocytes (Di), and pachytene primary spermatocytes (P), in seminiferous tubule. Stained with iron hematoxylin-orange G. X 1540.

Fig. 13. Maturing spermatids (MS), round spermatids (RS), spermatogonium (B), and zygotene primary spermatocyte (Z), in seminiferous tubule. Stained with iron hematoxylin-orange G. X 1540.



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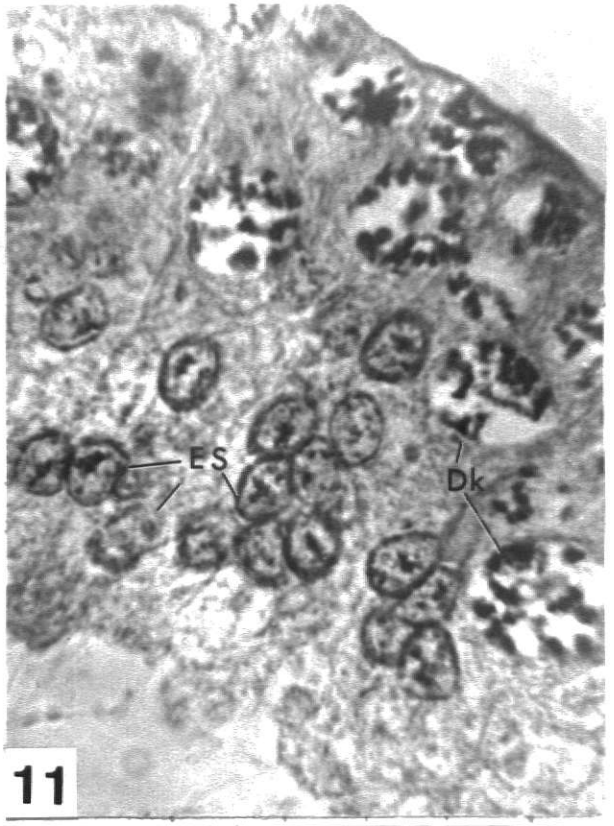

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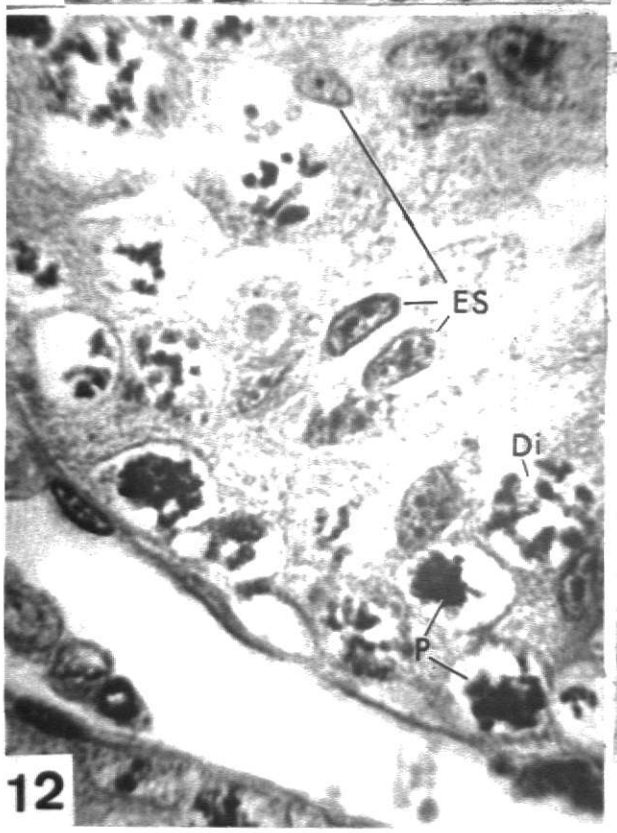

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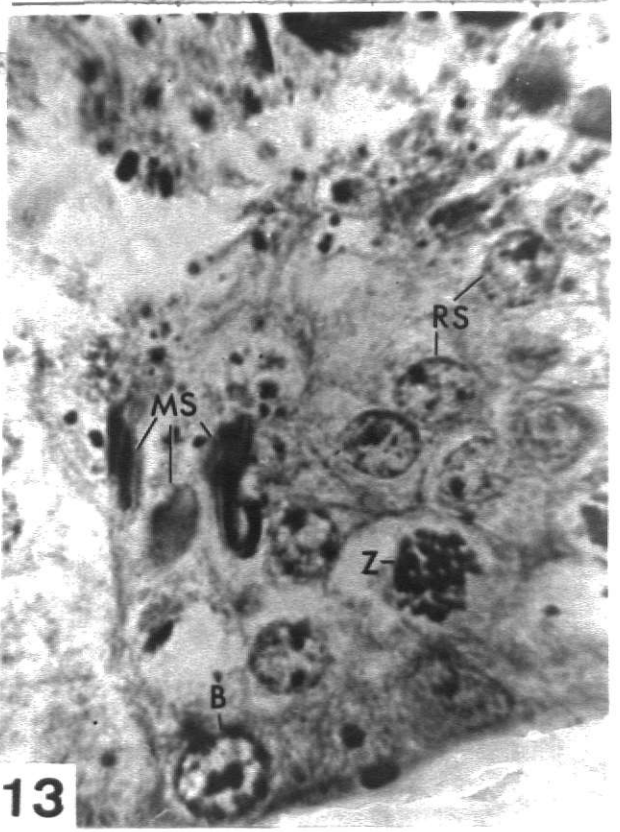

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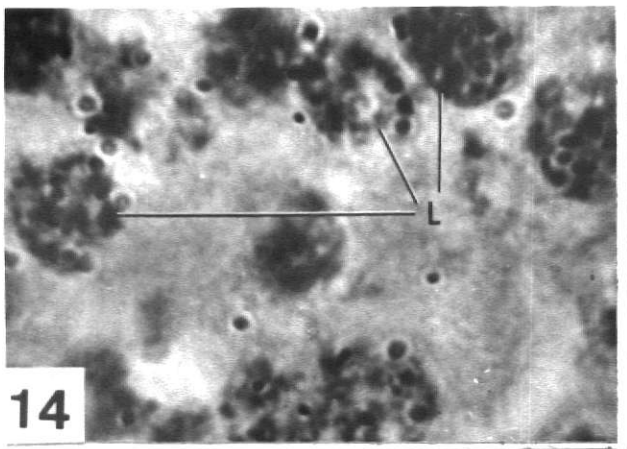
Fig. 14. Autoradiograph of leptotene primary spermatocytes (L). Exposure period 85 days. Stained with Harris' hematoxylin. X 2460.

Fig. 15. Autoradiograph of dictyate or diakinesis of primary spermatocytes (DK). Exposure period 85 days. Stained with Harris' hematoxylin. X 2460.

Fig. 16. Autoradiograph of round spermatids (RS). Exposure period 85 days. Stained with Harris' hematoxylin. X 2460.

Fig. 17. Autoradiograph of maturing spermatids (MS). Exposure period 85 days. Stained with Harris' hematoxylin. X 2460.

Fig. 18. Cells of seminiferous epithelium in chronological order of maturation: spermatogonium A (A), spermatogonia B (B), preleptotene primary spermatocytes (PL), leptotene primary spermatocytes (L), zygotene primary spermatocytes (Z), pachytene primary spermatocytes (P), early diplotene primary spermatocyte (Di), late diplotene primary spermatocytes (Di), round spermatids (RS), elongate spermatids (ES), maturing spermatids (MS). Stained with iron hematoxylin-orange G. X 1540.



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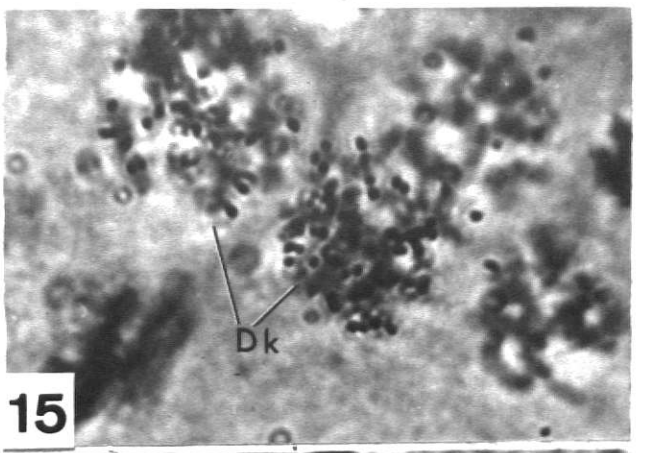

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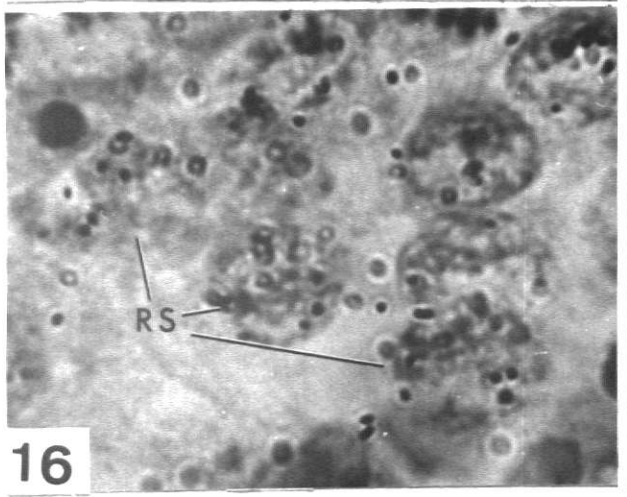

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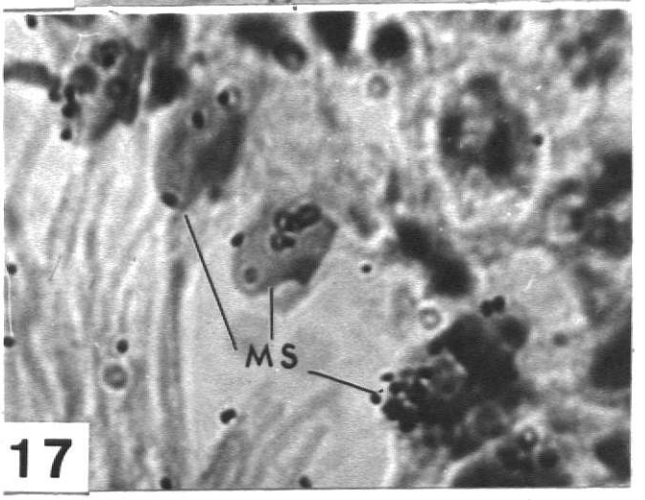

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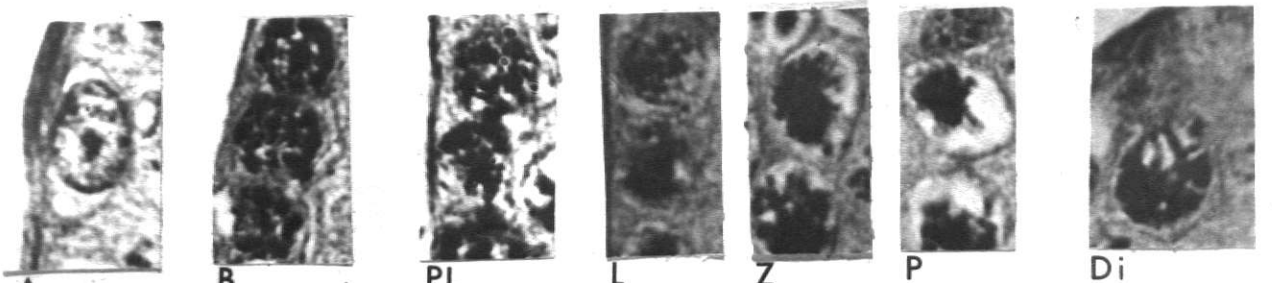

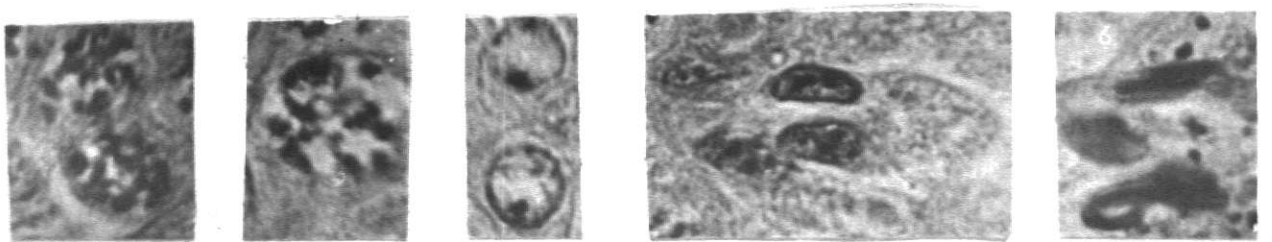

18

TABLE 1. RETAINED ACTIVITIES OF INDIVIDUAL TESTES PORTIONS

Animal number	Treatment groups	Hours from injection to sacrifice	Testes portions	Total wet weight of portions (mg)	Gross counts/ 20 minutes/ 30 mg tissue	DPM/mg tissue[a]	DPM/ total wet weight of testes portions[a]
19	VTA	0.25	LA	246.3	277428.	859.00	212000.
19	VTA	0.25	LP	203.1	83489.	261.00	53100.
19	VTA	0.25	RA	242.3	1558.	3.22	781.
19	VTA	0.25	RP	206.7	1477.	2.89	597.
21	VTA	0.25	LA	123.5	102322.	316.00	39000.
21	VTA	0.25	LP	117.9	37454.	113.00	13300.
21	VTA	0.25	RA	126.6	3382.	8.84	1120.
21	VTA	0.25	RP	113.6	3571.	9.42	1070.
23	VTA	1.	LA	88.3	205257.	645.00	57000.
23	VTA	1.	LP	94.9	142195.	458.00	43500.
23	VTA	1.	RA	85.6	3345.	8.97	768.
23	VTA	1.	RP	92.8	3239.	8.63	801.
24	VTA	1.	LA	231.9	122508.	389.00	90300.
24	VTA	1.	LP	192.2	49559.	154.00	29700.
24	VTA	1.	RA	229.9	3292.	8.80	2020.
24	VTA	1.	RP	199.9	3369.	8.93	1790.
20	VTA	24.	LA	101.0	60893.	181.00	18900.
20	VTA	24.	LP	89.7	51170.	155.00	13900.
20	VTA	24.	RA	72.9	1415.	2.70	197.
20	VTA	24.	RP	113.7	1377.	2.58	293.
26	VTA	24.	LA	123.3	50219.	157.00	19300.
26	VTA	24.	LP	114.3	10422.	31.20	3560.
26	VTA	24.	RA	141.3	2627.	6.41	906.
26	VTA	24.	RP	95.0	2687.	6.68	635.
17	VTA	48.	LA	183.8	142179.	446.00	82000.
17	VTA	48.	LP	187.3	91556.	287.00	53700.
17	VTA	48.	RA	164.3	1617.	3.36	552.
17	VTA	48.	RP	209.8	1718.	3.73	782.

TABLE 1., CONTINUED

Animal number	Treatment groups	Hours from injection to sacrifice	Testes portions	Total wet weight of portions (mg)	Gross counts/ 20 minutes/ 30 mg tissue	DPM/mg tissue[a]	DPM/total wet weight of testes portions[a]
18	VTA	48.	LA	116.3	89331.	280.00	32500.
18	VTA	48.	LP	173.0	40428.	126.00	21700.
18	VTA	48.	RA	170.4	1980.	4.55	776.
18	VTA	48.	RP	123.6	2072.	4.84	599.
27	VIP	0.25	LA	104.3	5515.	15.50	1610.
27	VIP	0.25	LP	98.5	3998.	10.70	1060.
27	VIP	0.25	RA	118.0	4439.	12.30	1450.
27	VIP	0.25	RP	97.7	4279.	11.70	1150.
28	VIP	0.25	LA	63.9	1930.	4.33	277.
28	VIP	0.25	LP	66.7	1861.	4.06	271.
28	VIP	0.25	RA	48.5	2125.	4.87	236.
28	VIP	0.25	RP	77.0	1622.	3.43	264.
29	VIP	1.	LA	128.2	3220.	8.46	1080.
29	VIP	1.	LP	86.5	3131.	8.06	697.
29	VIP	1.	RA	108.1	4045.	10.60	1150.
29	VIP	1.	RP	91.1	3403.	8.79	801.
30	VIP	1.	LA	66.1	5375.	15.00	993.
30	VIP	1.	LP	97.7	4146.	11.10	1080.
30	VIP	1.	RA	99.7	4741.	13.10	1300.
30	VIP	1.	RP	67.4	3765.	10.00	676.
31	VIP	24.	LA	98.4	3063.	7.97	784.
31	VIP	24.	LP	114.8	2510.	6.13	704.
31	VIP	24.	RA	112.8	2715.	6.87	775.
31	VIP	24.	RP	97.1	2321.	5.54	538.
32	VIP	24.	LA	119.3	2855.	7.31	872.
32	VIP	24.	LP	87.4	2780.	6.97	609.
32	VIP	24.	RA	99.6	2648.	6.75	672.
32	VIP	24.	RP	85.5	2488.	6.24	533.

TABLE 1., CONTINUED

Animal number	Treatment groups	Hours from injection to sacrifice	Testes portions	Total wet weight of portions (mg)	Gross counts/ 20 minutes/ 30 mg tissue	DPM/mg tissue[a]	DPM/total wet weight of testes portions[a]
33	VIP	48.	LA	162.3	853.	1.00	163.
33	VIP	48.	LP	121.8	893.	1.11	135.
33	VIP	48.	RA	138.6	880.	1.07	149.
33	VIP	48.	RP	149.4	942.	1.26	189.
34	VIP	48.	LA	102.9	1865.	4.19	431.
34	VIP	48.	LP	200.0	1719.	3.68	735.
34	VIP	48.	RA	146.9	1814.	4.03	592.
34	VIP	48.	RP	148.3	1706.	3.69	547.
10	VIT	0.25	LA	153.8	3755.	10.30	1580.
10	VIT	0.25	LP	148.3	734254.	2280.00	338000.
10	VIT	0.25	RA	136.4	1726.	3.75	512.
10	VIT	0.25	RP	155.5	1913.	4.28	665.
16	VIT	0.25	LA	111.8	3471.	9.25	1030.
16	VIT	0.25	LP	132.4	22826.	70.20	9300.
16	VIT	0.25	RA	125.6	2267.	5.38	675.
16	VIT	0.25	RP	128.8	2624.	6.48	835.
9	VIT	1.	LA	150.7	11308.	34.40	5180.
9	VIT	1.	LP	81.1	778172.	2450.00	199000.
9	VIT	1.	RA	133.8	2694.	6.89	922.
9	VIT	1.	RP	91.8	2441.	6.09	559.
14	VIT	1.	LA	161.7	51255.	160.00	25800.
14	VIT	1.	LP	123.9	606955.	1910.00	237000.
14	VIT	1.	RA	198.7	2649.	6.66	1320.
14	VIT	1.	RP	124.4	2495.	6.34	789.
12	VIT	24.	LA	209.9	42822.	135.00	28300.
12	VIT	24.	LP	149.2	230008.	723.00	108000.
12	VIT	24.	RA	194.1	2242.	5.45	1060.
12	VIT	24.	RP	158.6	2307.	5.58	886.

TABLE 1., CONTINUED

Animal number	Treatment groups	Hours from injection to sacrifice	Testes portions	Total wet weight of portions (mg)	Gross counts/ 20 minutes/ 30 mg tissue	DPM/mg tissue[a]	DPM/total wet weight of testes portions[a]
15	VIT	24.	LA	95.3	13457.	41.80	3390.
15	VIT	24.	LP	95.7	359600.	1160.00	111000.
15	VIT	24.	RA	86.7	1971.	4.59	397.
15	VIT	24.	RP	100.7	2362.	5.83	588.
11	VIT	48.	LA	149.7	12449.	37.50	5620.
11	VIT	48.	LP	165.6	149362.	469.00	77600.
11	VIT	48.	RA	175.6	1204.	2.08	365.
11	VIT	48.	RP	146.4	1144.	1.92	281.
13	VIT	48.	LA	182.7	2716.	6.77	1240.
13	VIT	48.	LP	177.6	207788.	635.00	113000.
13	VIT	48.	RA	175.6	2231.	5.27	925.
13	VIT	48.	RP	197.0	2040.	4.61	909.

a-values given to 3 significant figures

TABLE 2. MEAN[a] WEIGHTS OF TESTES AND TESTES PORTIONS FOR TREATMENT GROUPS AT TIMES OF SACRIFICE

Left anterior testes portions					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL[b]
VTA	185	160	112	150	152
VIP	84	97	109	133	106
VIT	133	156	153	166	152
ALL[c]	134	139	125	149	136

Right anterior testes portions					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	184	158	107	167	154
VIP	83	104	106	143	109
VIT	131	166	140	176	153
ALL	133	143	118	162	139

Left posterior testes portions					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	161	144	102	180	147
VIP	83	92	101	161	109
VIT	140	102	122	172	134
ALL	128	113	109	171	130

Right posterior testes portions					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	160	146	104	167	144
VIP	87	79	91	149	102
VIT	142	108	130	172	138
ALL	130	111	108	162	128

Left testes					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	345	304	214	330	298
VIP	167	189	210	294	215
VIT	273	259	275	338	286
ALL	262	251	233	320	266

Right testes					
Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	345	304	211	334	299
VIP	171	183	198	292	211
VIT	273	274	270	347	291
ALL	263	254	226	324	267

a-values given to nearest mg; n=2 for each time within each treatment group
b-mean value of all times within treatment group
c-mean value of all treatments within time

TABLE 3. MEAN[a] RETAINED ACTIVITIES (DPM)/MG TESTES AND TESTES PORTIONS FOR TREATMENT GROUPS AT TIMES OF SACRIFICE

Left anterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL[b]
VTA	588.00	517.00	172.00	363.00	410.00
VIP	9.89	11.70	7.64	2.60	7.97
VIT	9.77	97.10	88.40	22.20	54.40
ALL[c]	202.00	209.00	89.30	129.00	157.00

Right anterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	6.03	8.89	4.55	3.96	5.86
VIP	8.59	11.80	6.81	2.55	7.44
VIT	4.57	6.78	5.02	3.67	5.01
ALL	6.39	9.16	5.46	3.39	6.10

Left posterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	187.00	306.00	93.10	206.00	198.00
VIP	7.41	9.56	6.55	2.39	6.48
VIT	1170.00	2180.00	942.00	552.00	1210.00
ALL	456.00	832.00	347.00	254.00	472.00

Right posterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	6.16	8.78	4.63	4.29	5.96
VIP	7.61	9.41	5.89	2.48	6.35
VIT	5.38	6.22	5.71	3.27	5.14
ALL	6.38	8.13	5.41	3.34	5.82

Left testes

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	775.00	824.00	265.00	569.00	608.00
VIP	17.30	21.30	14.20	4.99	14.40
VIT	1180.00	2280.00	1030.00	574.00	1270.00
ALL	659.00	1040.00	437.00	383.00	630.00

Right testes

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	12.20	17.70	9.18	8.24	11.80
VIP	16.20	21.20	12.70	5.03	13.80
VIT	9.95	13.00	10.70	6.94	10.20
ALL	12.80	17.30	10.90	6.74	11.90

a-values given to 3 significant figures; n=2 for each time within each treatment group
b-mean value of all times within treatment group
c-mean value of all treatments within time

TABLE 4. MEAN[a] RETAINED ACTIVITIES (DPM) OF TESTES AND TESTES PORTIONS FOR TREATMENT GROUPS AT TIMES OF SACRIFICE

Left anterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL[b]
VTA	125000	73600	19100	57300	68800
VIP	944	1040	828	297	777
VIT	1310	15500	16200	3430	9100
ALL	42500	30100	12000	20300	26200

Right anterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	950	1400	551	664	890
VIP	844	1220	723	370	790
VIT	594	1120	728	645	772
ALL	796	1250	668	560	818

Left posterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	33200	36600	8740	37700	29100
VIP	665	889	656	435	661
VIT	174000	218000	110000	95200	149000
ALL	69100	85100	39600	44500	59600

Right posterior testes portions

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	834	1290	464	690	820
VIP	708	738	536	368	588
VIT	750	674	737	595	689
ALL	764	902	579	551	699

Left testes

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	159000	110000	27800	95000	97900
VIP	1610	1930	1480	732	1440
VIT	175000	233000	126000	98600	158000
ALL	112000	115000	51700	64800	85800

Right testes

Treatment groups	Hours from injection to sacrifice 0.25	1	24	48	ALL
VTA	1780	2690	1020	1350	1710
VIP	1550	1960	1260	738	1380
VIT	1340	1800	1460	1240	1460
ALL	1560	2150	1250	1110	1520

a-values given to 3 significant figures; n=2 for each time within each treatment group
b-mean value of all times within treatment
c-mean value of all treatments within time

TABLE 5. COMPARISONS OF MEAN[a] RETAINED ACTIVITIES (DPM) AMONG THE THREE TREATMENT GROUPS

Testes or portions	Treatment Groups VTA mean ± S.E.[b]	VIP mean ± S.E.[b]	VIT mean & S.E.[b]	Probabilities[c]
L	20.82 ± .54	12.76 ± .54	19.98 ± .54	2.97×10^{-7}
R	13.22 .42	12.68 .42	13.04 .42	6.62×10^{-1}
LA	10.83 .30	6.44 .30	8.41 .30	9.12×10^{-7}
LP	9.99 .34	6.32 .34	11.57 .34	5.07×10^{-7}
RA	6.62 .24	6.44 .24	6.56 .24	8.56×10^{-1}
RP	6.59 .19	6.24 .19	6.48 .19	4.28×10^{-1}

a-arithmetic means of $\log_e$-transformed data

b-standard error of the mean

c-probability, under the null hypothesis that treatment group means are equal, calculated using F statistics from analysis of variance (Morrison, 1967)

TABLE 6. COMPARISONS OF MEAN[a] RETAINED ACTIVITIES (DPM) BETWEEN TREATMENT GROUPS TAKEN TWO AT A TIME.

Testes or portions	Treatment groups mean ± S.E.[b]	Treatment groups mean ± S.E.[b]	Probabilities[c]
	VTA	VIP	
L	20.82 ± .54	12.76 ± .54	1.98×10^{-7}
R	13.22 .42	12.68 .42	3.81×10^{-1}
LA	10.83 .30	6.44 .30	2.91×10^{-7}
LP	9.99 .34	6.32 .34	6.81×10^{-6}
RA	6.62 .24	6.44 .24	5.90×10^{-1}
RP	6.59 .19	6.24 .19	2.11×10^{-1}
	VTA	VIT	
L	20.82 .54	19.98 .54	2.93×10^{-1}
R	13.22 .42	13.04 .42	7.64×10^{-1}
LA	10.83 .30	8.41 .30	9.17×10^{-5}
LP	9.99 .34	11.57 .34	7.17×10^{-3}
RA	6.62 .24	6.56 .24	8.41×10^{-1}
RP	6.59 .19	6.48 .19	6.79×10^{-1}
	VIP	VIT	
L	12.76 .54	19.98 .54	6.47×10^{-7}
R	12.68 .42	13.04 .42	5.58×10^{-1}
LA	6.44 .30	8.41 .30	5.02×10^{-4}
LP	6.32 .34	11.57 .34	1.58×10^{-7}
RA	6.44 .24	6.56 .24	7.32×10^{-1}
RP	6.24 .19	6.48 .19	3.87×10^{-1}

a-arithmetic means of $\log_e$-transformed data
b-standard error of the mean
c-probability under the null hypothesis that treatment group means are equal, calculated using F statistics from analysis of variance (Morrison, 1967)

TABLE 7. COMPARISONS OF MEAN[a] RETAINED ACTIVITIES (DPM) AMONG THE FOUR SACRIFICE TIMES WITHIN TREATMENT GROUPS

Treatment groups	Testes	Time from injection to sacrifice: 0.25 mean ± SE.[b]	1.0 mean ± SE.[b]	24.0 mean ± SE.[b]	48.0 mean ± SE.[b]	Probabilities[c]
VTA	L	21.6 ± 1.08	21.7 ± 1.08	18.7 ± 1.08	21.3 ± 1.08	0.217
VTA	R	13.5 ± .832	14.2 ± .832	12.1 ± .832	13.0 ± .832	0.375
VIP	L	12.8 ± 1.08	13.7 ± 1.08	13.2 ± 1.08	11.3 ± 1.08	0.471
VIP	R	12.7 ± .832	13.7 ± .832	12.8 ± .832	11.5 ± .832	0.340
VIT	L	18.1 ± 1.08	21.6 ± 1.08	20.9 ± 1.08	19.3 ± 1.08	0.145
VIT	R	13.0 ± .832	13.5 ± .832	13.1 ± .832	12.6 ± .832	0.892

a-arithmetic mean of $\log_e$-transformed data

b-standard error of the mean

c-probability, under the null hypothesis that treatment group means are equal, calculated using F statistics from analysis of variance (Morrison, 1967)

TABLE 8 CLASSIFICATION OF CELL TYPES COMPRISING THE SEMINIFEROUS EPITHELIUM IN M. OCHROGASTER AND THEIR COUNTERPARTS IN OTHER SPECIES AS DESCRIBED BY VARIOUS AUTHORS

Cell types	Cell types and stages in which they occur					
M. ochrogaster	rat[a]	mouse[b]	hamster[c]	guinea pig[d]	bull[e]	drake[f]
Spermatogonia						
Type A (A)	—	A:6	—	A:4	—	—
Type B (B)	B:4,5,6	B:5	—	B:5	—	—
Primary spermatocytes						
Preleptotene or resting (R)	R:7	—	—	R:7	Pl:5	—
Leptotene (L)	—	—	—	L:8	L:6	—
Zygotene (Z)	—	—	—	L:9 Z:10	Z:7	—
Pachytene (P)	—	—	—	Z:11	Z:8	L:1,2,5,6,7,8 Z:3
Diplotene (Di)	—	—	P:8-12	P:1-10,12	D:5	P:4,5
Dictyate or diakinesis (DK)	Di & Dk:13	—	Dk:13	—	Dk:7	P:7,8
Spermatocyte divisions (Sp.D.)	—	—	—	—	—	—
Spermatids						
Round (RS)	—	—	—	—	1	—
Elongate (ES)	—	—	—	8,9	5	—
Maturing (MS)	—	—	—	—	—	—

a-Daoust and Clermont, 1955
b-Oakbert, 1956
c-Clermont, 1954
d-Clermont, 1960
e-Gillette and Corwin, 1964
f-Clermont, 1958

TABLE 9. TIME REQUIRED FOR APPEARANCE OF LABEL IN CELL TYPES

Animal number (VTA)	Time from injection to sacrifice	Most mature cell type labeled
10	0.25 hrs	leptotene
17	1 hrs	leptotene
19	3 hrs	zygotene
25	6 hrs	zygotene
26	12 hrs	pachytene
23	18 hrs	pachytene
27	1. day	pachytene
24	2 days	pachytene
22	3. days	pachytene
21	4. days	pachytene
16	6 days	pachytene
15	7 days	diplotene
14	8 days	dictyate
11	9 days	dictyate
12	10 days	round spermatid
1	12 days	round spermatid
13	14 days	round spermatid
5	16 days	maturing, elongate, round spermatids
4	18 days	mature sperm; maturing and elongate spermatids
3	20 days	mature sperm, maturing spermatids
9	25 days	no labeled cells
8	30 days	no labeled cells
7	35 days	no labeled cells
6	40 days	no labeled cells

THE SPERMATOGENIC CYCLE OF MICROTUS OCHROGASTER AS DETERMINED BY AUTORADIOGRAPHIC TECHNIQUES

by

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ABSTRACT

The feasibility of direct injection of ^{3}H-dThd into the arterial blood supply of the testes of M. ochrogaster was studied by dissection and digestion of the testes following cardiac injection of latex, and by x-ray after cardiac injection of radio-opaque media. Although several anomalies of the arteries were found, injection of tracer was readily accomplished into the testicular artery on the ventral aspect of the posterior pole of the testis, allowing for first availability of the compound to the testis.

Three groups of eight sexually mature M. ochrogaster males were injected with 0.25 μc of ^{3}H-dThd intraperitoneally, intra-arterially, or into the testicular parenchyma to determine what percentage of the dose administered was retained by the testes. At 15 min, 1, 24, and 48 hr post-injection, two animals from each group were sacrificed, both testes from each animal were excised and cut into anterior and posterior portions.

Liquid scintillation samples were prepared from these 96 portions, counted, corrected for background and quenching, and the resultant data statistically analyzed. Of the administered doses the amounts retained by the testes averaged 0.51% for the intraperitoneally injected animals; 18% for the intra-arterially injected animals; and 28% for the intraparenchymally injected animals. Because of the low retention following intraperitoneal injections and the excessively high retention by the left posterior portion following intraparenchymal injection, the intra-arterial method which showed good distribution and non-toxic high levels of retention was adopted as the procedure to be used for injection of animals in which spermatogenesis was studied.

A classification system based on nuclear morphology was set up for the cells comprising the seminiferous epithelium in M. ochrogaster, by comparison

with descriptions given for other species.

Sexually mature males were injected with ^{3}H-dThd, then sacrificed at intervals from 15 min to 40 days, and autoradiographs prepared from histological preparation of testis tissue by dipping the slide in NTB-2 emulsion and incubating for 85 days.

Progression of the labeled front was determined as time after injection to be: leptotene, 25 min; pachytene, 12 hr; diplotene, 7 days; dictyate, 8 days; round spermatid 10 days; elongate sperm, 16 days; and mature sperm in the lumen, 18 days. No label was found after 20 days. The meiotic prophase lasts between 9 and 10 days; spermiogenesis is initiated between 9 and 10 days post-injection; and mature sperm free in the lumina were first detectable at 18 days post-injection.

Results indicate that prophase and spermiogenesis are shorter in M. ochrogaster than any other mammal described.