EARLY EFFECTS OF ABDOMINAL TEMPERATURE ON RAT TESTICULAR TISSUE: A HISTOLOGICAL BIOCHEMICAL AND ENZYMATIC STUDY

By

LARRY M. SCHANBACHER

Bachelor of Science

Northwestern State College

Alva, Oklahoma

1966

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1969

EARLY EFFECTS OF ABDOMINAL TEMPERATURE ON RAT TESTICULAR TISSUE: A HISTOLOGICAL BIOCHEMICAL AND ENZYMATIC STUDY

Thesis Approved:

Larry L. Ewing
Thesis Adviser

M. C. Morrissette

L E McDonald

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author is greatly indebted to his major professor, Dr. Larry L. Ewing, and wishes to express sincere appreciation for his guidance, his inexhaustible encouragement, his special interest and his personal assistance throughout the course of this study. Sincere appreciation is also expressed to Dr. Claude Desjardins for his assistance and many helpful suggestions during the construction of this manuscript. This appreciation is extended to the members of the faculty of the Department of Physiology and Pharmacology for their thoughtful suggestions and encouragements. The author is especially grateful to Dr. Bennie I. Osburn of the Department of Pathology for his assistance in assessing changes in testicular histology for this study. Special appreciation is extended to the author's present colleagues, Dr. V. K. Ganjam, Mr. J. A. Holt, Miss R. F. Clegg, Dr. W. Rhynes and other graduate students for their assistance and enjoyable company throughout this study. The author would also like to thank Mr. Robert Amey for his technical assistance during this study. For personal financial aid during this course of study, thanks is extended to the National Aeronautical Space Administration for a NASA Predoctorial Fellowship, Number NsG(T)-67-S3. Finally, and above all, a very special thanks to my wife, Connie, and my children, Allan and Yvonne, for their thoughtful sacrifices, dedicated love and encouragements. "Thank you for all of these."

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In many mammalian species the testes migrate from the body cavity and descend into the scrotum shortly before or after birth. By residing in the scrotum, the testes are provided with an environment that is 3-8^{o}C lower than the rest of the body (73). Failure of the testis to occupy its scrotal position irreversibly damages the spermatogenic and steroidogenic elements of the testis (96), indicating that the function of the scrotal testis is impaired at body temperatures.

In recent years, numerous studies have attempted to establish why normal testicular function only occurs at temperatures lower than the rest of the body. The majority of the studies that deal with abnormal testicular function at elevated temperatures have been concerned with quantitating biochemical and metabolic changes only after marked histological changes could be detected. One of the earliest cytological studies concerned with the effects of abdominal temperature on testicular germinal cell types demonstrated that histopathological changes could be detected as early as 48 hours after translocating the testes into the abdominal cavity (2). In spite of this cytological evidence, there have been few investigations on the biochemical and metabolic changes which precede histological alterations in testes translocated to the abdominal cavity.

In vitro metabolic studies on testicular metabolism following

testicular hyperthermia suggest that increased temperatures enhance anaerobic testicular metabolism (35,36). Thus, limiting supplies of substrate and oxygen to testicular tissue at abdominal temperatures could explain the deleterious effects of hyperthermia on the spermatogenic and steroidogenic elements of the testes (32). Comparative differences in the enzymatic activities of glucose metabolizing enzymes of abdominal (chicken) and scrotal (rabbit) testes support this concept. Based upon differences in the activity of glucose metabolizing enzymes in chicken and rabbit testes, an hypothesis has been suggested for the deleterious effects of abdominal temperature on mammalian spermatogenesis (3). According to the hypothesis, an increase in testicular temperature should modify the activity of selected glucose metabolizing enzymes prior to detecting histological alterations in the tissue. Therefore, the present study was designed to determine whether or not changes in selected glucose metabolizing enzymes and biochemical constituents preceded the histopathological alterations induced by relocating the testes in the abdominal cavity.

It was shown that the total activity of phosphofructokinase decreased 8 hours before that of hexokinase. The total activity of hexokinase dropped 36 hours before a decline in the soluble protein content was detected. Within 48 hours after the testes were placed in the body cavity abnormal germinal elements were noted. Histopathological observations indicated that abdominal temperature caused cellular disruption and disorganization of specific cell types during discrete stages of the spermatogenic cycle.

CHAPTER II

LITERATURE REVIEW

Histology of the Seminiferous Tubule

Bloom and Fawcett (4) indicate that the seminiferous epithelium consists of two distinct categories of cells. The first category includes the nutrient and supporting cells of Sertoli. The second category consists of the germinal or spermatogenic cells which, through proliferation and complex transformation, give rise to mature spermatozoa.

At the present time, the functional role of the Sertoli cells in the spermatogenic processes is questionable. Roosen-Rungi (87) determined that Sertoli cells occupy approximately 8.4% of the total testicular volume in rats which may suggest some physiological importance for these cells. Bloom and Fawcett (4) and Greep (45) indicate that numerous ultramicroscopic infoldings exist on the membrane surface of the Sertoli cells which are shaped to facilitate a close fitting for the heads of maturing spermatids. Since a large number of maturing spermatozoa are in close attachment with the Sertoli cells this attachment site could provide nutritional and supportive functions for maturing spermatozoa. However, since the two adjoining membranes between the Sertoli cells and the head of the maturing spermatozoa are not in intimate contact with each other, the nutritive role of this anchorage site is not fully understood. Nelson and Heller (77) have noted that the

quantity and distribution of glycogen and lipid in the Sertoli cells varies with spermatogenic activity in such a manner as to suggest that the maturing sperm consume these substances. These observations and those of Bloom and Fawcett (4) suggest that Sertoli cells provide the maturing spermatozoon with support as well as essential energy supplies. Some cases of infertility are thought to arise from deranged Sertoli cell function; that is, failure to promote the maturation of spermatozoa due to either a lack of sufficient attachment sites or too low nutritional supplies within the Sertoli cell. Sertoli cells are very resistant to noxious agents and the aging process. In comparison, cryptorchidism and toxic dibilitants such as the phenylthiazine tranquilizers and some barbiturates (39) completely destroy the germinal epithelium but these conditions scarcely affect Sertoli cells. Similarly, hypophysectomy results in a reduction of the seminiferous epithelium to one or two layers of non-functional germinal cells within 30 days. These remaining two layers are chiefly composed of Sertoli cells (47,92,93).

Although the Leydig cells are not part of the seminiferous tubule, their unique location relative to the seminiferous epithelium and its vascular supply suggests that these cells may play a role in either proliferating or maintaining the germinal epithelium. This suggestion is supported by observations which show that blood from the interstitial cells flows past the seminiferous tubules before entering the systemic circulation (1).

The mechanism whereby the spermatogonia differentiate and yet continue to renew their own stock has long been a perplexing matter. For many years it was assumed that germinal cell renewal occurred through a process termed differential mitosis (43). Thus, it was believed that

from the division of a spermatogonium, there arose one daughter cell that would differentiate as a spermatocyte and another that would remain as a primitive spermatogonium or stem cell. Detailed studies in monkies, Macacus rhesus, (18) and rats (17) have revealed that from a given mitosis, both daughter cells are destined to either differentiate or to remain as part of the stock of primitive spermatogonia. Hence, according to Clermont's (18) hypthesis, a single stem cell could give rise to eight primary spermatocytes, which is twice as efficient as the differential mitosis theory.

The formation of mature spermatozoa begins at stage VIII of the cycle of the seminiferous epithelium in the rat with the proliferation of spermatogonial type A dark (Ad) to type A pale (Ap) which subsequently differentiate into type B spermatogonium (19,45,64). Type B spermatogonia divide mitotically and give rise to primary spermatocytes. Primary diploid spermatocytes then transform into secondary spermatocytes by the process of meiosis. This reduction division involves the characteristic liptotene, zygotene, pachytene and diplotene steps of diakinesis. The secondary spermatocytes, now having a haploid number of chromosomes, undergo a mitotic-meiotic division to yield spermatids. Therefore, it is possible to receive sixty-four mature spermatozoa from one primitive type Ad spermatogonia (17).

Four cycles of 12 days each are required for the development of spermatogonia into free-swimming spermatozoa in the rat (53). Similarly four cycles of the seminiferous epithelium are required to produce free-swimming spermatozoa in mice (81), rams (82) and bulls (82). In contrast to rats, mice, rams and bulls, monkies require six complete cycles of the seminiferous epithelium to produce motile free-swimming

spermatozoa (18).

Studies on Artificial Cryptorchidism

Early investigations by Moore et al. (72,73) showed a temperature differential between the scrotum and the peritoneum ranging from 2°C in rabbits to 8°C in rats. Moore et al. (63,64,65,67,70,71) also demonstrated that placement of an active testis in the abdominal cavity for 60 days caused degeneration of the germinal epithelium in guinea pigs, rabbits, rats and sheep. Moore et al. (66,69) and Young (101) postulated that testicular degeneration was caused by elevated temperatures since either insulation or a single application of 47°C water for 30 minutes to the scrotum of guinea pigs caused involution of the seminiferous epithelium. Thus, these observations support the concept that there is an inverse relationship between germinal epithelium activity and testicular temperature.

More recently Asdell and Salisbury (2) noted histopathological changes in the germinal elements of rabbit testes within 48 hours after being exposed to abdominal temperatures. Electron microscopic studies indicate that temperature not only affects the germinal elements, but the connective tissue of the basement membrane as well. This basement membrane becomes distorted and separated from the cells making up the basal cell layer lining the seminiferous tubule (49). Therefore, elevated temperature may cause testicular degeneration by reducing the passage of nutritive material across the basement membrane, since there is no direct vascular supply to the germinal cells of the seminiferous tubules. Quantitative studies on the germinal cell types remaining in ram (75) and rat (10,13,14,80) testes after 2 weeks exposure to the

peritoneum suggests that abnormal testicular function was associated with the degeneration of maturing spermatids. This inhibitive influence on cell development arising from elevated temperature also resulted in a drastic reduction in the concentration of motile spermatozoa in the ejaculate (14).

Clegg (14) noted maximal degenerative changes in the seminiferous tubules of rats following 15 days of exposure of the testis to the abdomen. After 15 days, all germ cell types were reduced in number, spermatogonia being least affected while spermatids were affected the most. Spermatogonial mitosis and spermatocytial meiosis were also inhibited to some extent (14). If the testes are insulated or transferred to the abdominal cavity for 1 to 2 days, and then allowed to return to the scrotum, complete recovery of the germinal epithelium within 45 days may occur (68,76). However, if the testes are retained in the abdominal cavity for longer time periods (30 days or longer) the germinal elements may be permanently destroyed and complete loss of fertility will insue (5). Bowler (5), using anaesthetized male rats and immersing them up to the level of the penis in 43.5^{o}C water for 20 minutes suggests that the time for complete recovery of mature spermatozoa and fertility from a single application of heat had a mean of 60 days. However, repeated heat treatment for 20 minutes once every 6 weeks causes a delay in recovery of fertile spermatozoa, or a mean recovery time of 170 days. Bowler (5) suggested that the recovery time of 60 days for the single heat treated males corresponds well with the reported 52 day duration (15) of spermatogenesis in rats. Thus, it seems likely that recovery from a single exposure to 43.5^{o}C water takes place from stem cell spermatogonia. The mean recovery time may be prolonged some 110 days by

using repeated heat treatment suggesting that the stem cell spermatogonia may be permanently damaged (5).

Other studies (10,13,43) have shown that certain germinal cells are more resistant to heat than others with spermatogonia, spermatocytes and spermatids being resistant to thermal damage in that order. Exposing the testes to increased temperature (43°C) for 10 days causes the Sertoli cells to phagocytize unshed spermatogonia and spermatocytes. Chowdhurg (10), employing short term heat treatments (30 minutes in 43°C water for 2 days) and using Clermont's (19,53) classification for the stages of spermatogenesis in the rat, observed the damaging effects of increased testicular temperature on dividing spermatocytes to occur between stage IX (liptotene) and stage XIV of spermatogenesis. Leydig cell function did not appear to be altered by retaining the ram testis in the abdominal cavity for at least 10 days since no significant changes occurred in the fructose content, cell height and weight of the accessory sex glands (11). In a similar type of study no changes were observed in the fructose concentration of the semen after artificial cryptorchidism (12).

Davis and Firlit (24) studied the differences between "natural" and artificial cryptorchidism by comparing the morphological changes occurring in prepuberal and adult cryptorchid testes. The results of this study indicated that similar morphologic changes were observed in the seminiferous tubules for both the "natural" and artificial cryptorchid rats.

Theories on Spermatogenic Damage Due to High Testicular Temperature

During the last decade a number of investigations have been aimed at elucidating the mechanisms by which elevated temperatures damage the germinal epithelium. Cross and Silver (21) noted that oxygen tension increased following a brief 10 minute warming of the scrotal area in rabbits, dogs and sheep. Cross and Silver (21) suggested that these observations reflected an increase in metabolism of the tissue. In a similar experiment with rams, Free (40) attributed the increase in oxygen tension to purely physical effects of increased temperature on retention of blood gases within the testicular tissue. These results were more plausible since Free (40) accounted for the effects of temperature on the solubility of O_2 and CO_2 in the blood. Free (40) suggested that the decreased respiratory quotient (Q_{10}) associated with scrotal warming could be indicative of the qualitative changes in the metabolism of the heat treated testis. Waites and Setchell (98,99) have concluded from their experiments that testicular hypoxia resulting from increased testicular temperature is the causative factor for spermatogenic damage. In contrast to this interpretation, Fleeger and Van Demark (38) suggest that carbon dioxide might account for spermtogenic arrest due to the accumulation of this product at elevated temperatures. However, infertility has been noted in males exposed to external environments with low oxygen content (10% by volume (91)).

Ewing and VanDemark (33,34,35,36) and other investigators (8,22,23, 25,26,27,28,48,74) attribute spermatogenic damage caused by increased testicular temperature to an upset in glucose metabolism. This suggestion is supported by the early work of other investigators (29,30,31,84)

who have shown that the testis is more dependent on exogenous glucose than any other tissue studied. The demonstration that acute hypoglycemia causes severe damage to the germinal eipithelium in adult rats further supports the concept that the lack of substrate (glucose) results in testicular degeneration (58).

Baldwin and Ewing (3) compared the metabolic activites of rabbit (scrotal) and chicken (abdominal) testes and suggested a working hypothesis which may explain the deleterious effects of abdominal temperature on spermatogenesis in mammalian testes. The working hypothesis is based on the assumption that testicular blood flow is barely adequate to supply oxygen and glucose for metabolic processes in animals with scrotal testes. They hypothesized that high oxidative metabolism and low oxygen tension may favor glycolysis in chicken testes. In contrast, rabbit testes appear to metabolize glucose aerobically since oxygen would not be a limiting factor in scrotal testes. The observation that the activity of the hexomonophosphate shunt is low in the chicken testis may be compatable with the concept that most of the glucose-6-phosphate is being metabolized via the glycolytic pathway (3). In comparison to the chicken testis, the rabbit testis appears to have a lower oxidative metabolism possibly due to the lower temperature prevailing in the scrotum (32). This lower oxidative metabolism should lower the oxygen requirements for scrotal testes and oxygen would not be as limiting under these conditions. Therefore, phosphofructokinase would be blocked due to higher adenosine triphosphate (ATP) levels from reduced cofactors such as reduced nicotinamide adenine dinucleotide phosphate which shuttle electrons through the electron transport system causing glucose-6-phosphate to accumulate. An accumulation of glucose-6-phosphate may

reduce the activity of hexokinase since this product can inhibit the enzymatic activity of hexokinase. Consequently, glucose-6-phosphate would have to be metabolized via a different pathway in order to release some of the inhibition of hexokinase exerted by glucose-6-phosphate thus allowing glucose to be trapped within the cell. If testicular oxygen levels are barely adequate for glucose metabolism at scrotal temperatures, then glucose-6-phosphate could be metabolized via the hexomonophosphate shunt. By metabolizing glucose through the hexomonophosphate shunt, it would be possible to "detour" the block at phosphofructokinase by converting glucose-6-phosphate to glyceraldehyde-3-phosphate and this could either enter glycolysis or be recycled through the hexomonophosphate shunt. Under these circumstances the scrotal testis in rabbits could supply substrate for oxidative metabolism in spite of a reduction in glycolysis by inhibiting phosphofructokinase activity.

The hypothesis promulgated by Baldwin and Ewing (3) may explain differences in glucose metabolism by abdominal and scrotal testes and it could be used to explain the observations of Moore (68) that spermatogenesis ceases in a scrotal testis when it is exposed to elevated temperature. Therefore, exposing the scrotal testis to higher temperatures such as that of the body cavity may increase oxidative metabolism and concomitantly reduce oxygen tension in the extracellular fluid of the testis. Thus, an elevation in testicular temperature may demand more glucose than the testicular circulation can furnish the testis. This near anaerobic state stimulates glycolysis by releasing ATP's inhibition on phosphofructokinase. Stimulation of phosphofructokinase activity releases the inhibition on hexokinase by removing the glucose-6-phosphate which was normally shunted through the hexomonophosphate

shunt under aerobic conditions. These circumstances create an additional demand for glucose which is barely adequate to satisfy normal metabolic processes and consequently the substrate becomes rate limiting and the increased requirements for ATP may not be met. Thus, spermatogenic arrest insues at elevated temperature due to the lack of sufficient oxygen and glucose to the tissue.

LeVier (54) studied some of the effects of temperature on several metabolic pathways in rat testes incubated in vitro. The results of this work suggest that glucose may be preferentially metabolized via the hexomonophosphate shunt rather than the Embden-Meyerhof pathway when the tissue was maintained at 32°C (scrotal temperature). These observations imply that the production of reduced nicotinamide adenine dinucleotide may be impaired at temperatures above 32°C since a reduction in the activity of the hexomonophosphate shunt would reduce the formation of ATP and this could release the block on phosphofructokinase. Therefore, LeVier (54) concluded when the testes are exposed to temperatures that are above normal, glycolysis may be favored and glucose may eventually become rate limiting in rat testicular tissue. Baldwin and Ewing (3) arrived at the same conclusion with their enzyme studies and suggested a similar hypothesis for glucose metabolism in rabbit testes.

Means and Hall (61,62) added further support for the necessity of exogenous glucose supplies for the production of high energy compounds such as ATP for stimulating protein biosynthesis in rat testicular tissue incubated in vitro. Means and Hall (62) concluded that the concentration of ATP was approximately one-third (1.50 μM ATP/g wet tissue) lower than normal when exogenous glucose was excluded from the incubation media. Testicular protein content was reduced in the absence of

glucose, but when glucose was added to the media, the specific activity of protein increased 2 to 4 times above control values. This observation confirms the findings of Davis and Morris (26), that stimulation of protein biosynthesis by glucose in the testis is a unique function of this organ. Thus, it appears that protein biosynthesis in the testis is directly related to the level of ATP and glucose.

Regulation and Metabolism of Glucose as a Primary Energy Source for the Scrotal Testes

In this section, the role of five different glucose metabolizing enzymes will be considered. These enzymes include: hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase. Hexokinase and phosphofructokinase are the two regulatory enzymes of glycolysis, while pyruvate kinase is the rate limiting enzyme associated with glycolysis. Glucose-6-phosphate dehydrogenase is a branch point enzyme from glycolysis which initiaties the oxidative metabolism of glucose leading to the hexomonophosphate shunt. Lactate dehydrogenase converts the end-product of glycolysis, pyruvate, to lactic acid when the citric acid cycle is not operating.

Hexokinase traps glucose within the cell and forms the phosphorylated intermediate, glucose-6-phosphate. Rose and O'Connell (88) demonstrated that the rate of utilization of glucose was inversely related to the concentration of glucose-6-phosphate. Other findings by Rose *et al.* (89) have indicated that inorganic phosphate will reduce the inhibition of glucose-6-phosphate on hexokinase. Thus, a quantitative effort to monitor this enzyme's activity would allow one to predict the efficiency of this enzyme to bring glucose into the cell.

The second regulatory step in glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. This reaction is catalyzed by phosphofructokinase and this enzyme may be inhibited by high levels of ATP (52,56,59,60,83,86). The inhibitory effect of ATP on phosphofructokinase may be reversed by increasing the concentration of 5'-adenosine monophosphate (5'-AMP), 3',5'-cyclic AMP, adenosine diphosphate (ADP), fructose-6-phosphate, and inorganic phosphate (60). Under aerobic conditions the level of ATP would be higher than normal and this could inhibit phosphofructokinase activity resulting in higher than normal levels of glucose-6-phosphate and lower than normal levels of hexokinase activity (79,83,86,97,100). Consequently, under anaerobic conditions, glucose would be primarily metabolized via the hexomonophosphate shunt since the concentration of glucose-6-phosphate would be markedly increased. Initiating the pentose phosphate pathway should minimize the product inhibition on hexokinase so that additional glucose could be trapped within the cell. Pyruvate kinase could still convert phospho(enol)pyruvate to pyruvate, even though the first portion of glycolysis was not functioning because pyruvate could be derived from glyceraldehyde-3-phosphate via the hexomonophosphate shunt.

Baldwin and Ewing (3) have indicated that in the rabbit tesis the aerobic pathway or the hexomonophosphate shunt is the major pathway for supplying energy and catabolism of glucose. Increasing the temperature of the scrotal testes from 36 to 39^{o}C causes a shift from aerobic to anaerobic glucose metabolism which may result from inadequate oxygen supplies (3). If the temperature in the testicular tissue is increased, then there would seem to be a greater demand for energy which could only be met through glycolysis. Since a glycolytic metabolism does not

provide sufficient energy to support the germinal epithelium of abdominal testes (11,12), then something must occur to the enzyme associated with this pathway. By measuring the total and specific activities of the five enzymes mentioned, it could be possible to determine the relationships of each enzyme with the metabolism of glucose in the normal and cryptorchid testis.

CHAPTER III

MATERIALS AND METHODS

Materials

Chemicals

A complete list of chemicals used in this work, their type or grade, and the company from which they were purchased may be found in Appendix A. The procedures that were used to prepare the reagents needed for this study are described in Appendix B. Care was taken to avoid selection of chemicals containing substances that could inhibit enzyme activity (i.e., high levels of ATP or contaminating enzymes). The soluble noninhibitive sodium and potassium salt derivatives were used as soon as possible after being purchased in order to minimize interfering breakdown products so that the proper molar concentration of the desired chemical could be achieved.

Animals

Sexually mature male Sprague-Dawley rats, weighing 300 $\pm$ 5 g were purchased from the Holtzman Company, Madison, Wisconsin at 70 to 80 days of age. The rats were housed alone in stainless steel cages in an animal room maintained at 22^{o}C with 12 hours of light every 24 hours. The

animals were provided with water and Purina Laboratory Chow[1] ad libitum. All animals were allowed to adjust to these conditions for 1 week before being used in the present study.

Methods

Surgical Procedures

The animals were anesthetized with ether (anesthesia grade) and the hair was clipped from the scrotal and pelvic area. The surgical field was swabbed with 95% ethanol and an incision (1 cm long) was made on both sides of the sheath of the penis so that each testis could be forced anteriorly through the inguinal canal and into the abdominal cavity. Following this, the muscular tissue lining the inguinal canal was pulled anteriorly through the incision in order to place a suture through this tissue. The suture (size 000 silk thread) was not allowed to pass through the testis, epididymis or vascular supply and the gubernaculum was left intact. The abdominal muscles were sutured and the superficial incision made in the skin was closed with a wound clip.

After both testes were placed in the abdominal cavity, pressure was applied posteriorly on the abdominal wall in an attempt to force the testes into the scrotum in order to check the completeness of the operation. Sham operated rats were similarly treated, except that the suture thread was only passed through the inguinal canal muscle layer and not tied. All animals were inspected for intact sutures when they were killed. The main advantage of this procedure over suturing the testis

---

[1]Ralston Purina Company, St. Louis, Missouri.

directly to the abdominal wall was that testicular tissue was not damaged by the suture material.

Rats were killed at 0, 2, 4, 8, 12, 24 and 48 hours after the testes were retained in the abdominal cavity. A similar procedure was followed for the sham operated animals. A total of 98 rats were subjected to bilateral abdominal translocation of the testes and 21 rats were sham operated. A total of 7 animals were used for histological studies.

Histological Techniques

Rat testes were placed in Bouins' solution for 30 minutes after they were retained in the abdominal cavity for either 0, 2, 4, 8, 12 or 48 hours. Only one animal was used per time interval. Following this, each pair of testes were sectioned transversely into 5mm slices to facilitate fixation and the tissues were allowed to remain in the fixative an additional 12 hours. Tissues were prepared for sectioning by impregnating them with Paraplast[2] under vacuum. Sections (5μ) were cut using a Spencer Model 820 microtome[3] and stained with either hematoxylin-eosin (H&E) or periodic acid-Schiff's leuco-fuchsin (PAS) and counter stained with eosin. Microphotographs were taken in order to compare the stages of the cycle of the seminiferous epithelium.

Tissue Preparation

Each animal was sacrificed by a sharp blow at the base of the skull. The testes were removed and immediately placed in 0.154 M KCl at

---

[2]Scientific Products, Evanston, Illinois.

[3]American Optical, Buffalo, New York.

5°C. The tunics were removed from each testis and both testes were blotted dry and weighed to the nearest milligram. After weighing, both testes were placed in a 15 ml Ten Broeck[4] glass homogenizer (No. 7727) containing isotonic KCl (0.154 M) at 5°C. A 20% homogenate (w/v) was prepared by adding four volumes of 0.154 M KCl to each gram of testicular tissue. The pestle was consistantly moved up and down 12 times, counting up and down as one complete stroke. An aliquot of this homogenate was frozen at -20°C and saved for nucleic acid determination.

The remaining homogenate (12 ml) was transferred to a Beckman No. 302235 cellulose nitrate tube and centrifuged at 105,000 g for 60 minutes at 4°C in a Beckman Model L-2 preparative ultracentrifuge[5]. This procedure removed the interferring microsomal ATPase's and subcellular particles. The supernatant material obtained after centrifugation consisted of a transparent fluid with an overlying opaque lipid layer. The transparent layer was carefully removed with a 10 ml syringe fitted with a 6 inch 18 gauge blunt stainless steel needle and transferred into dialysis tubing (Union Carbide No. 20)[6]. Usually seven dialysis bags each containing 7 ml of the 105,000 g supernatant fluid were dialyzed against 2,000 ml of 0.154 M KCl for 10 hours at 4°C. Following this, each dialyzed preparation was transferred to a 10 ml test tube and kept on ice until assayed. The entire tissue preparation procedure was completed in a cold room maintained at 4°C to insure maximal enzyme

---

[4]Corning, Houston, Texas.

[5]Scientific and Process Instruments Division, Beckman Instruments, Inc., Fullerton, California.

[6]Scientific Products, Evanston, Illinois.

activity and to reduce the metabolic activity within the 105,000 g supernatant.

Colorimetric Assays

Three quantitative colorimetric assays were performed using a Bansch and Lamb Spectronic 20[7]. These determinations included the quantitation of soluble protein from the dialyzed preparation as well as the ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of the homogenized tissue. The soluble protein was determined by the method of Lowry et al. (57). Ribonucleic acid content was measured by the procedure outlined by Ceriotti (9) while DNA was quantitated according to the method described by Burton (7). Schneider's nucleic acid extraction procedure (90) was modified by adding dioxane to the trichloroacetic acid (TCA) precipitate as suggested by Sripati (94) and by increasing the concentration of TCA in the initial precipitation from 10 to 15% as suggested by Steele (88). These modifications permitted approximately 100% of the nucleic acids to be extracted from the tissue.

Figure 1 illustrates the modified extraction procedure of Schneider (90) for extracting nucleic acids.

Spectrophotometric Assays

The procedures developed by Baldwin and Ewing (3) were used to measure the enzyme activity of homogenates prepared from rat testicular tissue. In preliminary experiments, the following conditions were established for each enzyme assay: 1) optimum substrate concentration,

---

[7]W. H. Curtin and Company, Dallas, Texas.

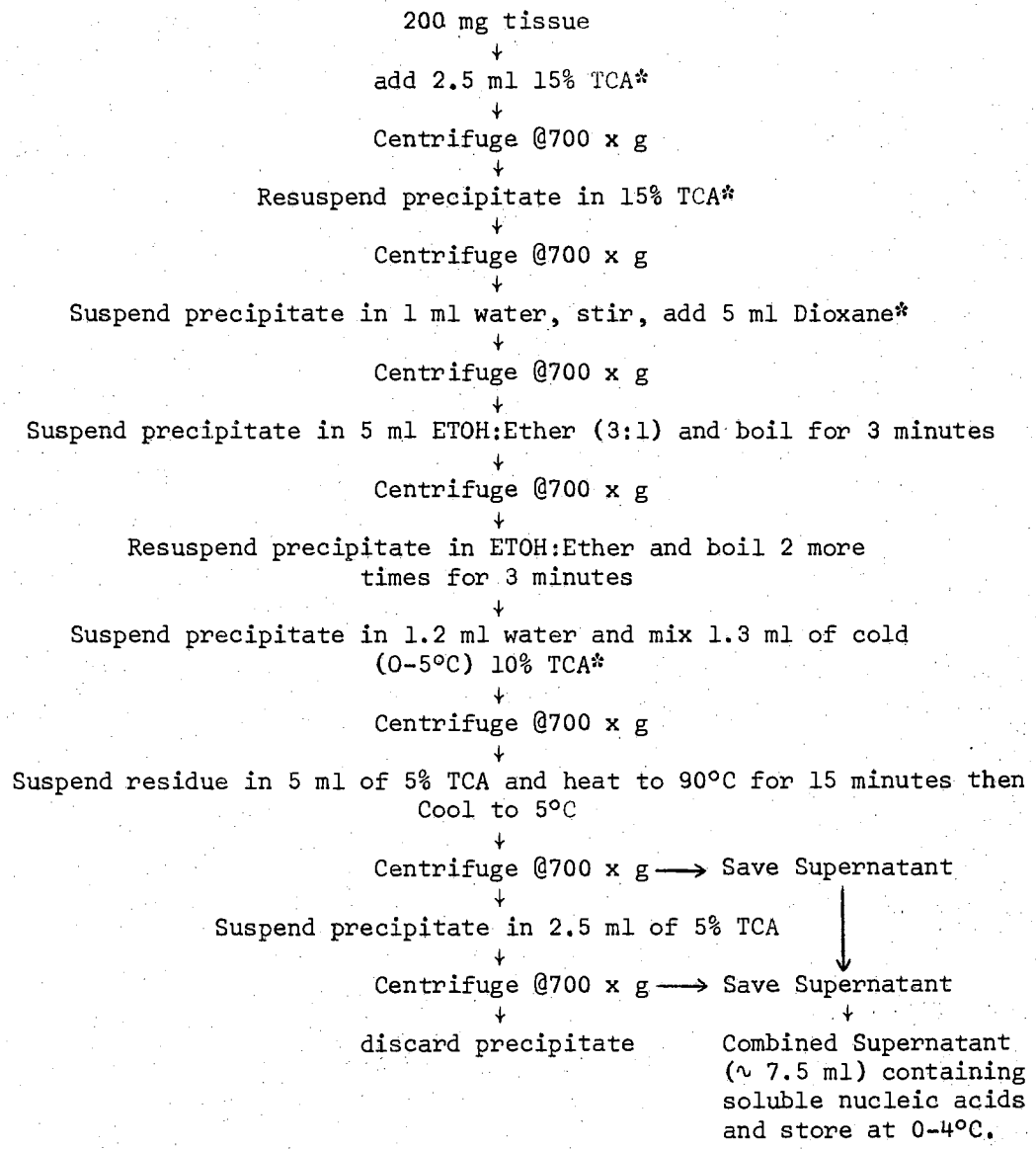


Figure 1. Modified Technique for Extracting Nucleic Acids From 200 mg of Testicular Tissue Used in This Study (* indicates points which were modified from Schneider's (90) procedure.)

substrate concentration that gave a zero order reaction; 2) optimum pH, pH at which the enzyme performed at its maximum rate (phosphofructokinase, pH 9.0; hexokinase, glucose-6-phosphate dehydrogenase and pyruvic kinase, pH 8.0; lactate dehydrogenase, pH 7.5) and 3) optimum enzyme concentration, a concentration that would give a measurable activity, and also would exhibit a straight line relationship when plotted against the velocity (see Figure 2). All enzyme activities were measured at 340 nm by recording the rate of change in absorbance of the cofactor associated with each reaction for 3 minutes at 25°C. A Cary Model 15 recording spectrophotometer[8] equipped with a Lauda Model K-2/R temperature regulator[9] was used in these studies. The buffers for each enzyme assay were made according to the procedure described by Gomori (44). Dilution of dialyzed preparation with non-buffered glass distilled water was eliminated due to a loss of enzymatic activity which was encountered in preliminary experiments. The straight line portion of each enzymatic reaction was used to calculate the enzymatic activity, giving the change in optical density per minute. The activities for each enzyme were expressed either as total activity (μ moles cofactor/g tissue/minute) or as specific activity (μM cofactor/mg soluble protein/minute).

The sequence for pipetting reagents was to add buffer first, dialyzed enzyme preparation second and substrate last. The cuvette containing the reaction mixture was placed in the spectrophotometer after the substrate was added to initiate the reaction. All cuvettes were washed in a vacuum operated cuvette washer with soapy water, rinsed with glass

---

[8]Applied Physics Corporation, Monrovia, California.

[9]Brinkmann Instruments, Westbury, New York.

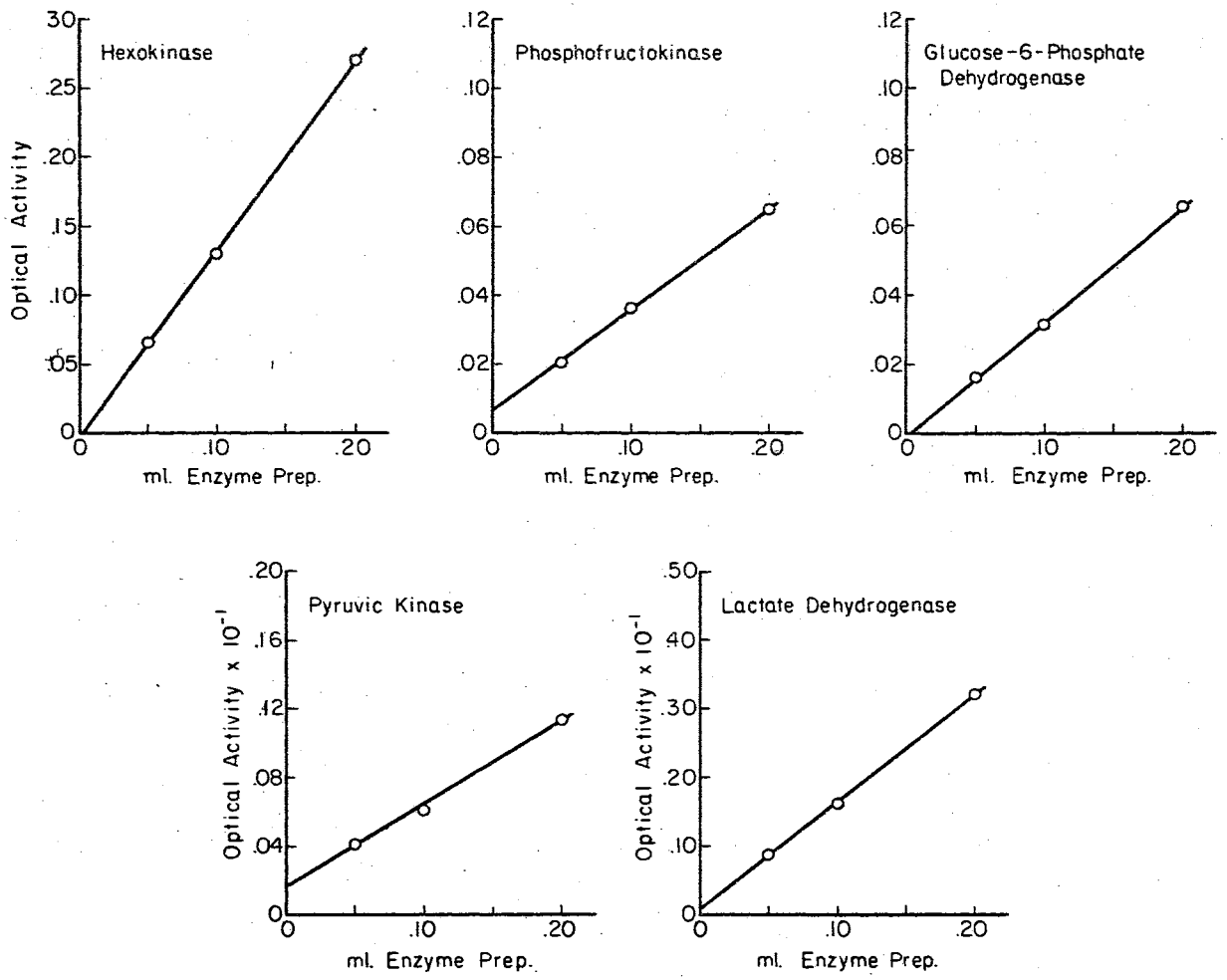


Figure 2. Enzyme Concentration Versus Changes in Enzyme Activity (Optical Activity)

distilled water and dryed with acetone.

Enzyme Assays

The following enzyme assays were carried out to assure maximum enzyme activities: a) Hexokinase was measured by coupling the reaction with glucose-6-phosphate dehydrogenase. The prcedure was similar to that described by Hannon and Vaughan (46) and Baldwin and Ewing (3) and consisted of the following: 100 μM Tris buffer, pH 8.0; 20 μM $MgCl_2$; 5 μM ATP; 0.6 μM nicotinamide adenine dinucleotide phosphate (NADP); 1.0 unit of glucose-6-phosphate dehydrogenase (G-6-P DH); 0.1 ml of the dialyzed enzyme preparation; and 10 μM glucose to initiate the reaction. Total volume was 3.0 ml. The reduction of NADP was followed for a period of 4 minutes with the 3 minute straight-line portion of the curve used to determine enzyme activity. b) Glucose-6-phosphate dehydrogenase (G-6-P DH) activity was measured by a modification of the procedure by Kornberg and Horecker (51) and Baldwin and Ewing (3) which consisted of the following: 100 μM Tris buffer, pH 8.0; 10 μM $MgCl_2$; 10 μM iodoacetate, pH 7.2; 0.6 μM NADP; 0.1 ml of dialyzed enzyme preparation; and 5.0 μM glucose-6-phosphate to initiate the reaction in the sample cuvette. The reference cuvette contained all ingredients except glucose-6-phosphate. Clock and McLean (41) have shown that the catalytic activity of this enzymatic reaction is the sum of the reduced NADP produced by reactions catalyzed by glucose-6-phosphate and by 6-phosphogluconate dehydrogenase. c) Phosphofructokinase activity was measured by coupling the enzyme with aldolase and α-glycerophosphate dehydrogenase to measure the disappearance of reduced nicotinamide adenine dinucleotide (NADH). The procedure used for measuring this

enzyme's activity was patterned after that described by Ling et al. (55) and Baldwin and Ewing (3) which consisted of the following reaction mixture: 100 μM Tris buffer, pH 9.0; 1.0 μM $MgCl_2$; 20 μM cysteine; 5 μM ATP; 0.6 μM NADH; 0.2 ml each of aldolase (1:50 dilution) and α-glycerophosphate dehydrogenase (1:50 dilution); 0.1 ml of dialyzed enzyme preparation (diluted 1:1); and 20 μM of fructose-6-phosphate was added to start the enzymatic reaction. Total volume was 3.0 ml. In this enzyme assay the reaction rate was nonlinear if smaller amounts (less than 20 μM) of fructose-6-phosphate were used. The reference cuvette contained all ingredients except fructose-6-phosphate. The activity was measured through the coupled oxidation of NADH by α-glycerophosphate dehydrogenase. d) Pyruvate kinase was measured by the method of Bucher and Pfleiderer (6) and Baldwin and Ewing (3). Lactate dehydrogenase was used as a coupling enzyme for this reaction. The assay consisted of the following: 100 μM triethanolamine-HCl buffer, pH 8.0; 75 μM $MgCl_2$; 75 μM KCl; 5 μM ADP; 0.2 μM NADH; 0.1 ml lactate dehydrogenase (1:100 dilution); 0.1 ml of dialyzed enzyme preparation (1:50 dilution); and 10 μM of phospho(enol)pyruvate were added to initiate the reaction sequences. The total volume was 3.0 ml. Using the trisodium salt of phospho(enol)pyruvate eliminated the preincubation period which was necessary in Baldwin and Ewing's (3) assay procedure. The oxidation of NADH was measured using the coupled enzyme reaction of lactate dehydrogenase. e) Lactate dehydrogenase was assayed by measuring the oxidation of NADH. Essentially the assay procedure was adopted from the methods of Kornberg (50) and Baldwin and Ewing (3) which consisted of the following: 100 μM phosphate buffer, pH 7.5; 0.4 μM NADH; 0.1 ml of dialyzed enzyme preparation (1:75 dilution); and the

reaction initiated with the addition of 1.0 μM pyruvate. The total volume was 3.0 ml.

Statistical Design and Analysis

The experiments were arranged in a completely randomized block design consisting of seven treatment groups with 3 to 14 replicates per treatment, depending on the experiment and criteria measures. All data were subjected to analysis of variance (see Appendix C) and when found to be significant, differences in treatment means were detected by Duncan's New Multiple-Range Test (95).

CHAPTER IV

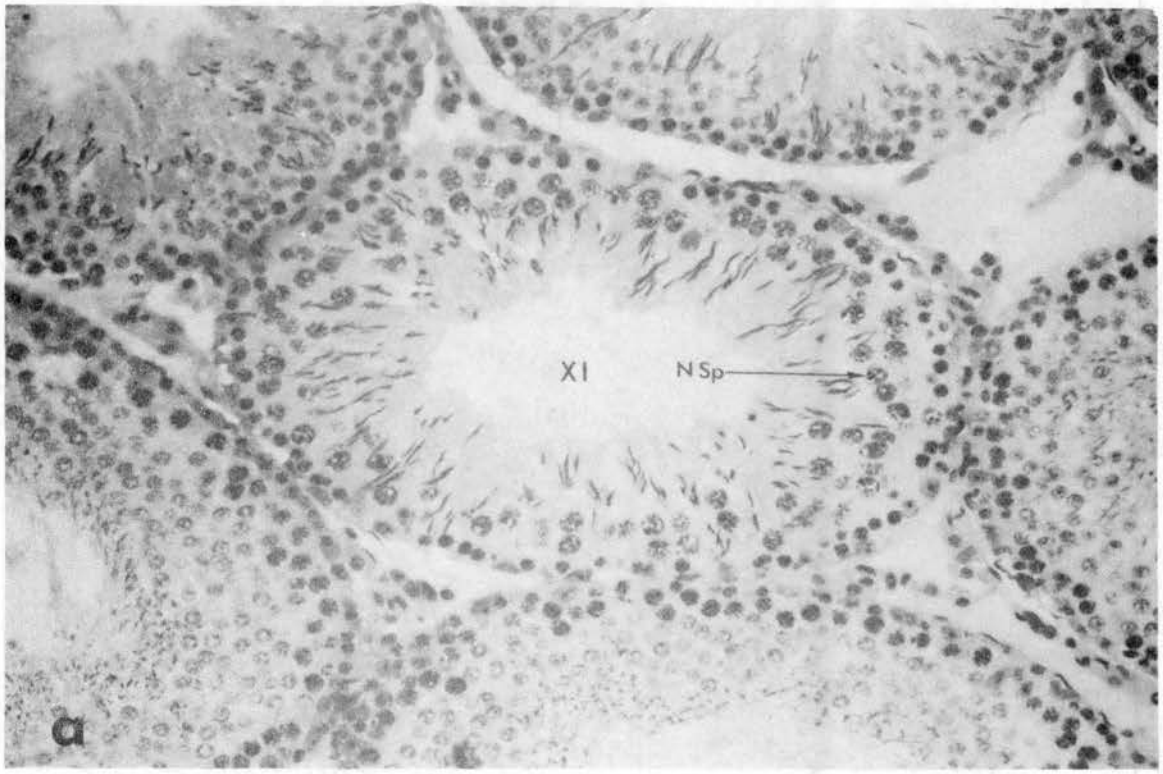
RESULTS

The objectives of this study were: 1) to determine the time at which histopathological changes occur in rat testes exposed to abdominal temperature, 2) to characterize any abnormalities among the seminiferous tubules associated with degenerating testicular tissue and 3) to determine whether biochemical or enzymatic changes precede the histological abnormalities which are induced by placing the testes in the body cavity.

Histopathological Findings

Abnormalities of spermatogenic cells in the seminiferous epithelium noted after 48 hours exposure to abdominal temperature are shown in Figures 3 and 4. Sections of testicular tissue stained with H&E (Figure 3) illustrate the difference in staining density of the cytoplasm (eosin-positive) and the nuclear material (hemotoxylin-positive) by comparing the histological features of the control group (Figure 3a) with those of the 48 hour treatment group (Figure 3b). The sections of testicular tissue stained with PAS and counter stained with eosin (Figure 4) show positive staining relationships between connective tissue, cellular membranes and nuclear material which cannot be observed with H&E staining. Figures 3a and 4a show normal mitotic activity (NSp) in the seminiferous tubules of the sham operated animals. All stages of

Figure 3. A Histological Comparison Using Hematoxylin-eosin Staining of the Response of the Seminiferous Epithelium in Rat Testis Subjected to Abdominal Temperature. a) Control testis 48 hours after sham operation, normal spermatocytes (NSp); b) Experimental Testis 48 hours after exposure to abdominal cavity, degenerating spermatocytes (DSp). Roman numerals represent the approximate stage of spermatogenesis in the designated seminiferous epithelium. (X 280).


XI
N Sp
a

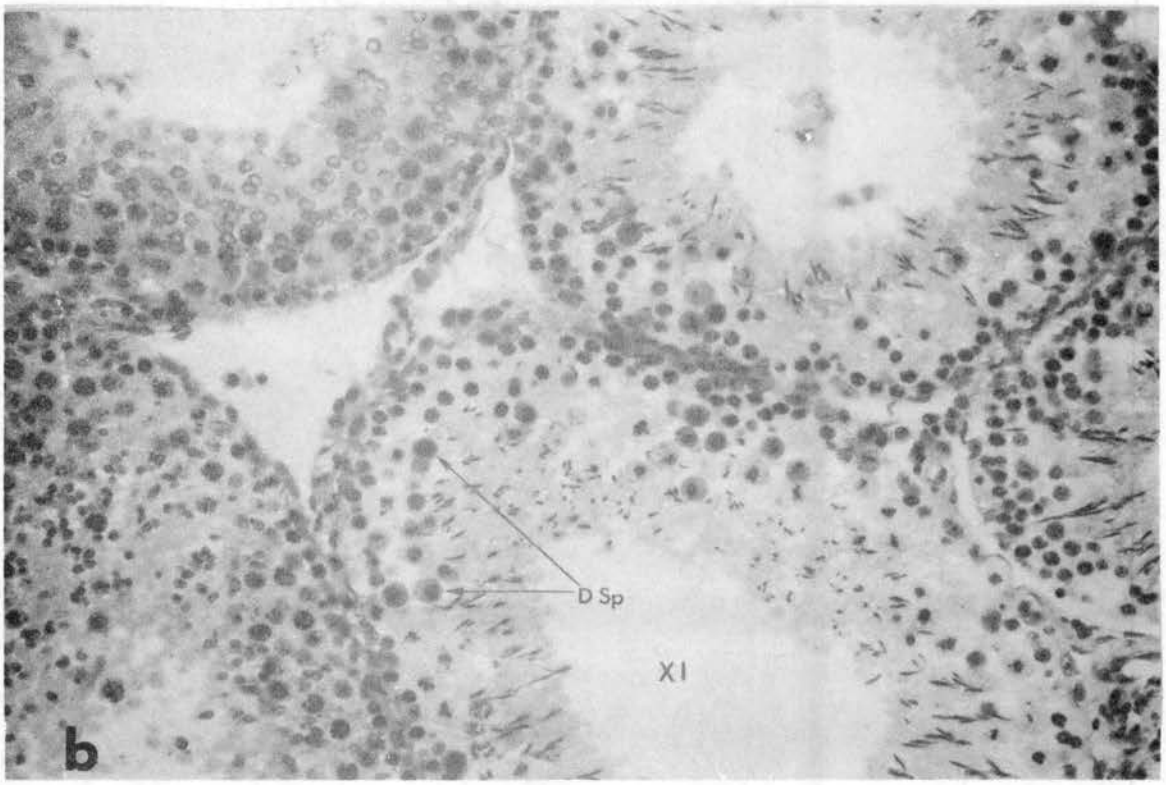
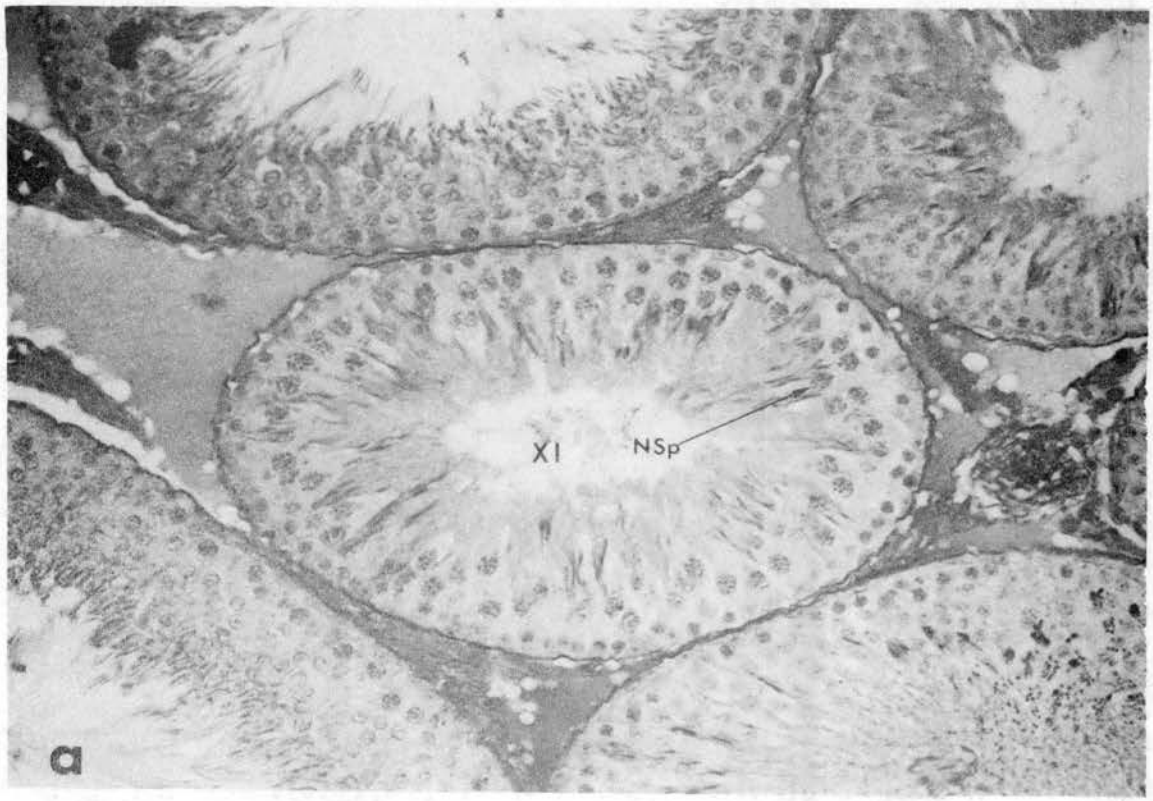
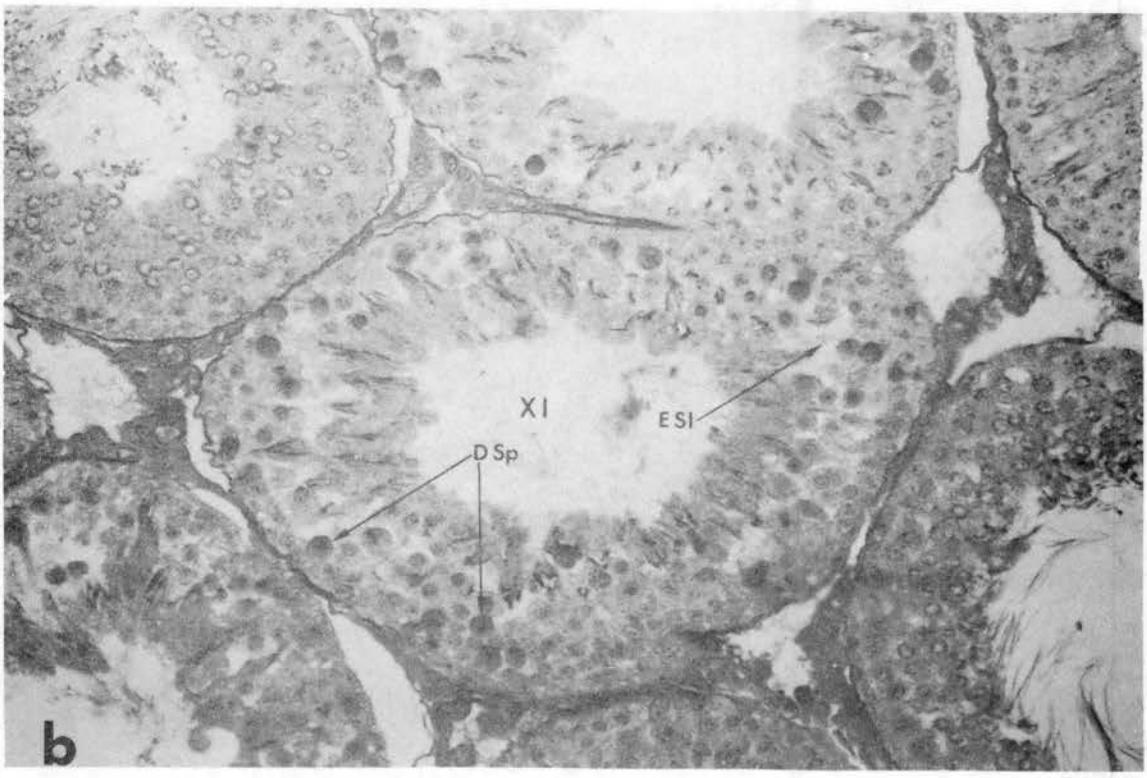

D Sp
XI
b

Figure 4. A Histological Comparison Using Periodic Acid-Schiff's Leucofuchsin Staining of the Response of the Seminiferous Epithelium in Rat Testis Subjected to Abdominal Temperature. a) Control testis 48 hours after sham operation, normal spermatocytes (NSp); b) Experimental testis 48 hours after exposure to abdominal cavity, degenerating spermatocytes (DSp), epithelium sloughing (ES1). Roman numberals represent the approximate state of spermatogenesis in the designated seminiferous tubules. (X 280)


XI
NSp
a


XI
ESI
DSp
b

the seminiferous epithelium were represented with distinct spermatogonia, primary and secondary spermatocytes and maturing spermatids being present. Forty-eight hours after the sham operation it was not possible to detect any histological changes in the seminiferous epithelium surrounding cross-sectional tubules of Figures 3a and 4a.

The degenerative effects of heat on the germinal cells lining the seminiferous epithelium are shown in Figures 3b and 4b. The degenerative cells (DSp), indicated by arrows, were characterized by large, hematoxylin-positive nuclei and eosin-positive cytoplasm as shown in Figure 3b, as well as the PAS-positive cells (DSp) shown in Figure 4b. The nuclei of these cells were usually eccentric and enlarged. These degenerating cells were identified as primary spermatocytes seemingly progressing through meiotic diakinesis. Degeneration of diakinetic spermatocytes after 48 hours of abdominal temperature seems to be specifically associated with stages IX through XIII of spermatogenesis, since stages other than stages IX through XIII of spermatogenesis, do not contain degenerating spermatocytes as seen in the seminiferous epithelium of surrounding cross-sectional tubules of Figure 3b and 4b respectively. Other evidence for specific degenerative changes within testes exposed to abdominal temperature is shown in Figure 4b by the gaps or spaces appearing in the cells of the seminiferous epithelium, marked by an arrow (ES1). Some of the degenerative cells that appear in Figure 4b show little PAS-positive nuclear material and appear to be "ghost cells". Although these degenerative conditions can be seen very distinctly after 48 hours exposure, some signs of degeneration were seen as early as 24 hours after translocating the testis to the abdominal cavity. Thus, exposure of the testis to abdominal temperature causes a

definite degeneration of the seminiferous epithelial cells which occurs sometime between 24 and 48 hours during stages IX through XIII of spermatogenesis and this involves the meiotic division of primary and secondary spermatocytes.

Effects of Sham Operation on the Weight, Soluble Protein and Enzymatic Activity of Rat Testicular Tissue

Table I shows the effect of sham operation on the weight and soluble protein content of rat testes at various time periods after sham operation. Analysis of variance indicated that the sham operation did not cause any significant changes ($P>0.10$) in either the weight or the soluble protein content of the testes.

The effect of sham operation on the total and specific activities of selected glucose metabolizing enzymes in rat testicular tissue is summarized in Table II. Analysis of variance failed to show any significant changes ($P>0.25$) in either the total or specific activities of hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase following the sham operation.

Effects of Abdominal Temperature on the Weight, Biochemical Composition and Enzymatic Activity of Rat Testicular Tissue

Table III shows the effects of abdominal temperature on the weight and biochemical composition of the testes. Translocating the testes to the abdominal cavity for various time periods up to 48 hours had no measurable effect on testicular weight ($P>0.10$). Hyperthermia did not change the quantity of deoxyribonucleic and ribonucleic acid present in testicular tissue ($P>0.10$). However, the soluble protein content of the

TABLE I

EFFECT OF SHAM-OPERATION ON THE WEIGHT AND SOLUBLE PROTEIN CONTENT OF RAT TESTES

Criteria Measured	Hours After Sham Operation						
	0	2	4	8	12	24	48
Paired Testis Weight (g)	3.34[a] ±.07	3.14 ±.15	3.15 ±.20	2.97 ±.08	3.36 ±.10	3.19 ±.06	3.00 ±.21
Soluble Protein (mg/g)	28.6 ±2.3	28.4 ±4.2	30.4 ±2.2	28.9 ±1.7	29.2 ±2.2	29.6 ±1.8	29.3 ±1.0

[a]Each value represents the mean ± standard error of three rats.

TABLE II

EFFECT OF SHAM-OPERATION ON THE MEAN TOTAL AND SPECIFIC ACTIVITY OF SELECTED GLUCOSE METABOLIZING ENZYMES IN RAT TESTICULAR TISSUE

Enzyme	Type of Activity	Hours After Sham Operation						
		0	2	4	8	12	24	48
Hexokinase	Total	1.67[a] ±.06	1.69 ±.10	1.76 ±.03	1.68 ±.04	1.67 ±.01	1.63 ±.05	1.59 ±.06
	Specific[b]	5.91 ±.51	5.88 ±.93	5.83 ±.35	5.88 ±.44	5.77 ±.47	5.55 ±.32	5.43 ±.26
Phosphofructokinase	Total	0.47 ±.01	0.52 ±.04	0.50 ±.05	0.54 ±.06	0.55 ±.06	0.52 ±.06	0.51 ±.06
	Specific[b]	1.70 ±.10	1.80 ±.25	1.70 ±.28	1.80 ±.12	1.90 ±.05	1.80 ±.24	1.80 ±.26
Glucose-6-Phosphate Dehydrogenase	Total	0.40 ±.03	0.42 ±.04	0.44 ±.03	0.40 ±.02	0.40 ±.02	0.39 ±.02	0.38 ±.03
	Specific[b]	1.40 ±.08	1.49 ±.30	1.46 ±.19	1.39 ±.09	1.37 ±.14	1.32 ±.05	1.31 ±.12
Pyruvate Kinase	Total	7.09 ±.58	7.22 ±.23	6.69 ±.35	7.36 ±1.3	7.62 ±.83	8.17 ±1.6	6.29 ±.71
	Specific[c]	2.53 ±.34	2.49 ±.27	2.23 ±.25	2.60 ±.55	2.65 ±.37	2.72 ±.34	2.14 ±.18
Lactate Dehydrogenase	Total	33.1 ±1.4	31.7 ±1.3	28.9 ±3.3	31.7 ±2.0	31.7 ±2.8	33.5 ±2.0	33.1 ±2.6
	Specific[c]	1.16 ±.04	1.11 ±.18	0.95 ±.11	1.10 ±.02	1.09 ±.11	1.15 ±.12	1.14 ±.13

[a]Each value represents the mean ± standard error of three rats.

[b]Specific activity x 10^{-2}.

[c]Specific activity x 10^{-1}.

TABLE III

EFFECT OF ABDOMINAL TEMPERATURE ON THE WEIGHT AND CHEMICAL COMPOSITION OF RAT TESTES

Criteria Measured	No. of Animals	Hours of Exposure to Abdominal Temperature						
		0	2	4	8	12	24	48
Paired Testis Weight (g)	6	3.4[a] ±.15	3.4 ±.07	3.2 ±.07	3.2 ±.07	3.2 ±.12	3.3 ±.08	3.3 ±.05
DNA (mg/g)	14	2.3 ±.04	2.4 ±.05	2.3 ±.05	2.4 ±.04	2.3 ±.05	2.3 ±.06	2.3 ±.06
RNA (mg/g)	14	3.0 ±.05	3.0 ±.08	3.0 ±.05	3.0 ±.05	3.0 ±.09	3.0 ±.07	2.9 ±.05
Soluble Protein (mg/g)	15	29.5 ±.72	29.5 ±.84	27.6 ±.75	28.3 ±.69	29.0 ±.75	27.7 ±.84	26.9** ±.74

[a]Each value represents the mean ± standard error.

**($P<0.01$).

testes dropped significantly ($P<0.01$) from an average of 29.5 to 26.9 mg/g between 0 and 48 hours after exposing the testes to abdominal temperature.

Table IV summarizes the effect of abdominal temperature on the mean total and specific activity of glucose-6-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase on rat testicular tissue. Neither the total nor the specific activities of each of these enzymes were influenced by exposing the testes to abdominal temperature for periods as long as 48 hours. In contrast to these findings, Figure 5 shows that the total activity of hexokinase increased initially and declined gradually as the length of time the testis was exposed to abdominal temperature increased. Comparison of differences in treatment means of total hexokinase activity showed no appreciable changes between the 0, 2, 4 and 8 hour treatment groups ($P>0.10$). However, total hexokinase activity noted at 12 and 24 hours after exposure to abdominal temperature was significantly ($P<0.05$) lower than the 0, 2, 4 and 8 hour treatment groups. The total activity of hexokinase detected in the 48 hour treatment group was the only value that was significantly ($P<0.05$) lower than the 0 hour control group.

Figure 6 shows that the total activity of testicular phosphofructokinase was depressed within the first 4 hours of temperature elevation. Statistical analysis revealed that the total activity of phosphofructokinase in the 4, 8, 12, 24 and 48 hour treatment groups was significantly ($P<0.05$) lower than the 0 hour treatment group. There was no detectable difference in total phosphofructokinase between the 0 and 2 hour treatment groups ($P>0.05$).

The decline in total phosphofructokinase activity was similar to

TABLE IV

EFFECT OF ABDOMINAL TEMPERATURE ON THE MEAN TOTAL AND SPECIFIC ACTIVITY OF SELECTED GLUCOSE METABOLIZING ENZYMES IN RAT TESTES

Enzyme Activity	No. of Animals	Type of Activity	Hours of Exposure to Abdominal Temperature						
			0	2	4	8	12	24	48
Hexokinase	7	Specific[b]	5.44 ±.39	5.84 ±.44	5.45 ±.35	5.50 ±.46	5.23 ±.37	5.34 ±.41	5.06 ±.44
Glucose-6-Phosphate Dehydrogenase	7	Total	0.36[a] ±.03	0.40 ±.03	0.43 ±.02	0.40 ±.02	0.41 ±.03	0.38 ±.02	0.36 ±.04
		Specific[b]	1.23 ±.10	1.31 ±.10	1.34 ±.10	1.29 ±.10	1.26 ±.10	1.28 ±.10	1.21 ±.17
Pyruvate Kinase	5	Total	5.54 ±.53	5.38 ±.49	5.46 ±.40	4.42 ±.59	5.04 ±.35	4.89 ±.45	4.49 ±.33
		Specific[c]	1.93 ±.02	1.87 ±.01	1.83 ±.01	1.51 ±.02	1.72 ±.01	1.80 ±.01	1.58 ±.01
Lactate Dehydrogenase	6	Total	32.1 ±1.4	30.6 ±1.0	31.1 ±1.3	29.8 ±1.4	33.0 ±1.9	29.6 ±1.6	30.5 ±1.8
		Specific	1.11 ±.09	1.08 ±.04	1.08 ±.06	1.03 ±.05	1.14 ±.07	1.10 ±.07	1.09 ±.07

[a]Each value represents the mean ± standard error.

[b]Specific activity x 10^{-2}.

[c]Specific activity x 10^{-1}.

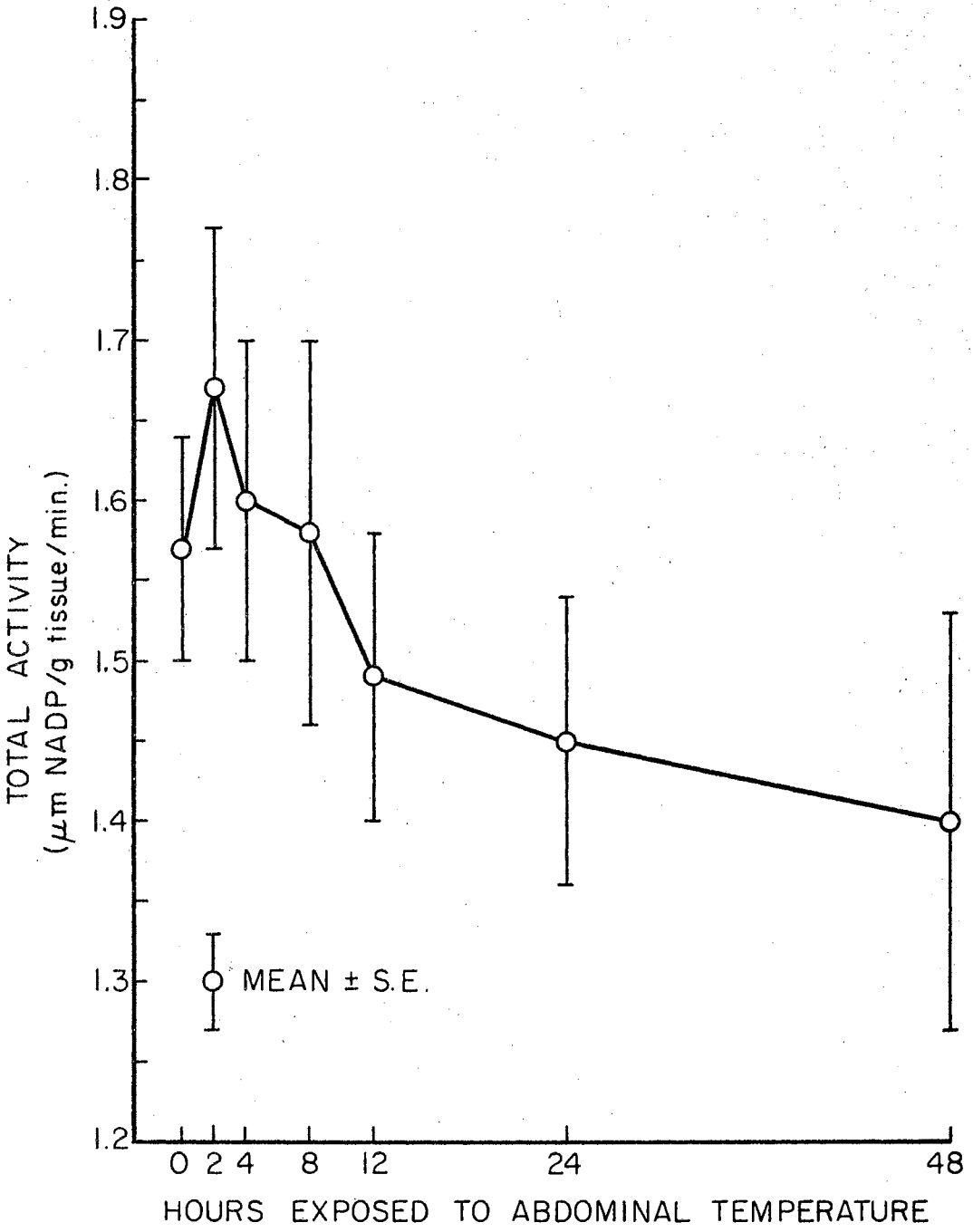


Figure 5. Effect of Abdominal Temperature on the Total Activity of Hexokinase in Rat Testicular Tissue

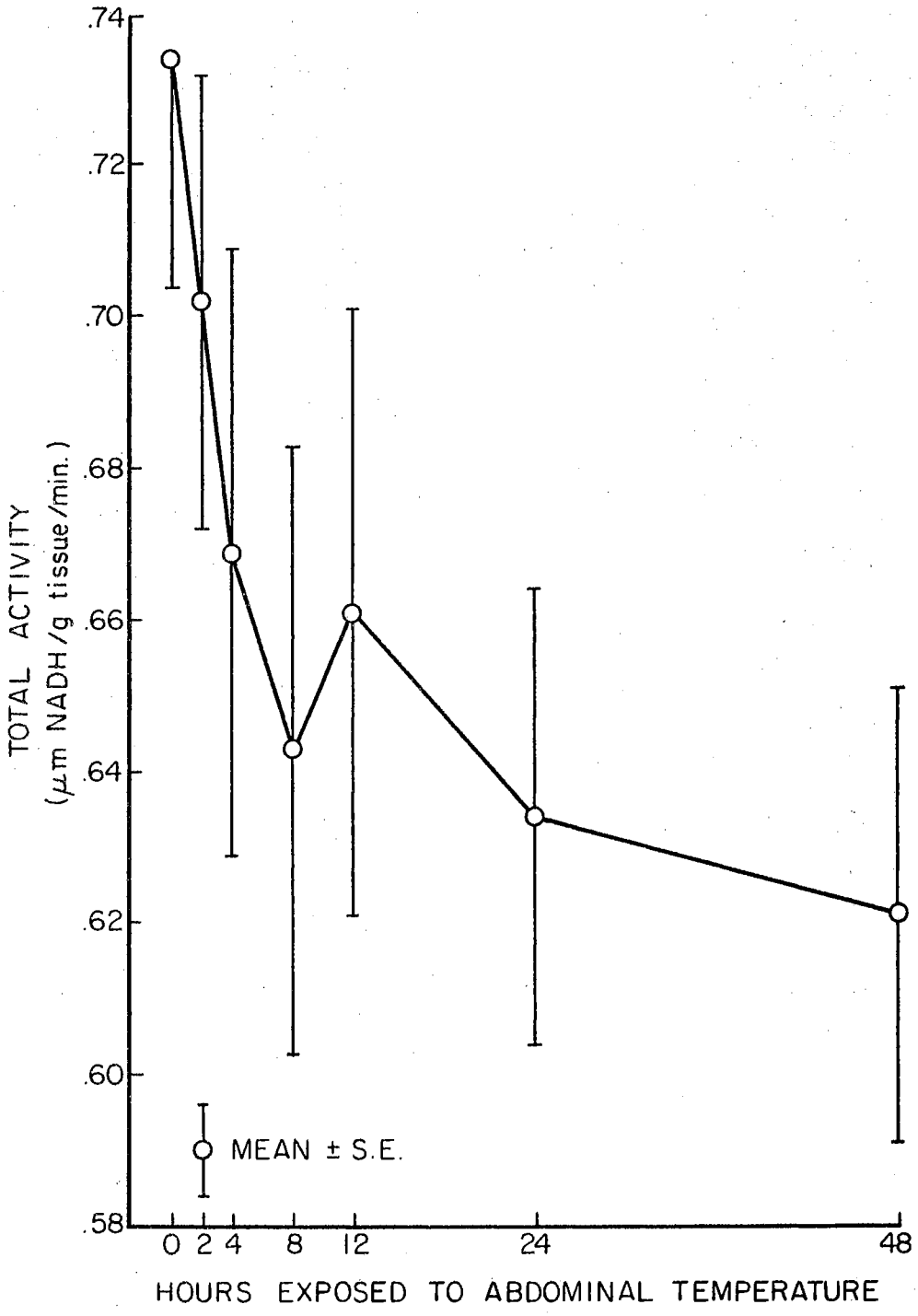


Figure 6. Effect of Abdominal Temperature on the Total Activity of Phosphofructokinase in Rat Testicular Tissue

the decline in specific phosphofructokinase activity through the 48 hour period that the testes were exposed to abdominal temperature. The specific activity of phosphofructokinase (Figure 7), dropped from 2.5 to 2.2×10^{-2} μM NADH/mg protein/minute between 0 and 8 hours after translocating the testes to the abdomen ($P<0.05$). The initial drop in specific activity noted during the first 8 hours was followed by a slight rise and subsequent decline so that the changes in specific activity tended to parallel those in total activity. The reduction in the specific activity of phosphofructokinase between 0 and 48 hours ($P<0.05$) suggests that the activity of this enzyme may be decreasing faster than the soluble protein content of the testis.

A summary of these data show the following: 1) abdominal temperature interrupted the spermatogenic processes at stages IX through XIII sometime between 24 and 48 hours after translocating the testes in the abdomen, 2) sham operation had no effect on the weight, soluble protein content and the activity (total and specific) of the five glucose metabolizing enzymes measured in rat testicular tissue, 3) translocation of the testis to the abdomen altered the level of soluble proteins but it did not effect the specific activity of hexokinase or the total or specific activities of glucose-6-phosphate dehydrogenase, pyruvate kinase or lactate dehydrogenase, 4) the total activity of phosphofructokinase dropped 8 hours before noting a decline in hexokinase activity and 5) only the specific activity of phosphofructokinase was affected after 48 hours exposure to abdominal temperature.

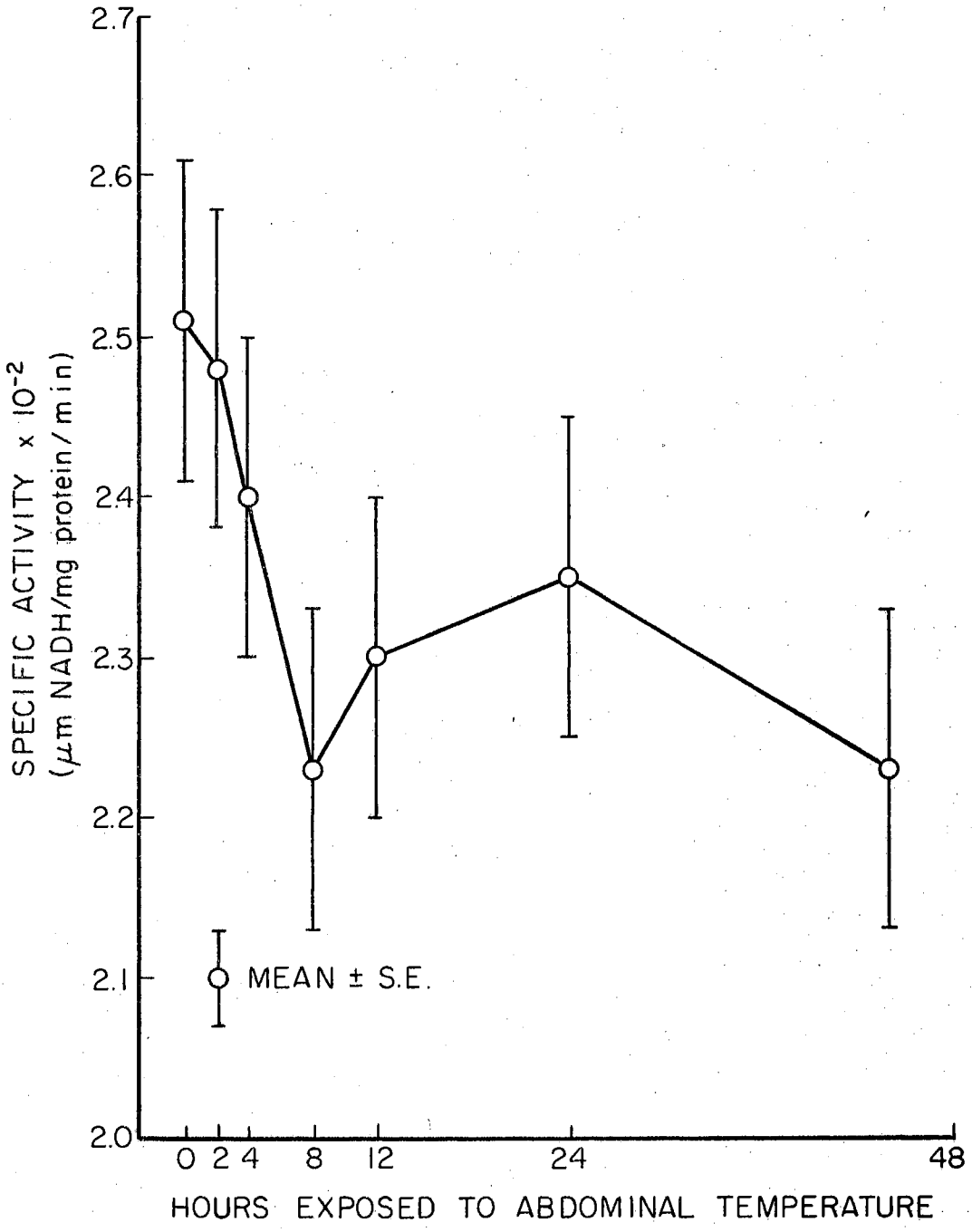


Figure 7. Effect of Abdominal Temperature on the Specific Activity of Phosphofructokinase in Rat Testicular Tissue

CHAPTER V

DISCUSSION

The results of this study demonstrated that distinct histological alterations involving degeneration of primary and possibly secondary spermatocytes occurred sometime between 24 and 48 hours following exposure of the testes to the abdominal cavity. Only tubules which appeared to be in stages IX through XIII of the spermatogenic cycle were affected by translocating the testes to the abdominal cavity. All other germinal cells, spermatogonia, spermatids and spermatozoa, appeared to be histologically normal. These observations suggested that increased testicular temperature interrupts meiotic processes sometime during stages IX through XIII of spermatogenesis.

The effects of elevated temperature on the germinal elements of the seminiferous epithelium do not coincide with reported histological findings after hypophysectomy. In hypophysectomized rats, only the spermatogonia and spermatocytes seem to be maintained while the remaining cell types slough off into the luminal space (1). In comparison to these observations, the present findings indicate that degenerating spermatocytes and the other cell types remain intact and attached to the adjoining germinal cell layers and to the basement membrane.

Elevated testicular temperature is not unique in causing degeneration of primary spermatocytes in stages IX through XIII of spermatogenesis. Among other agents that cause primary spermatocyte degeneration

are derivatives of the antibacterial drugs, the nitrofuran and dinitropyrrole compounds. The nitrofuran derivatives inhibit glucose utilization (85) and cause arrest of spermatogenesis at the primary spermatocyte stage (78). The biochemical basis for the effect of dinitropyrrole compounds on testicular tissue is unknown, but in view of the early histochemical changes observed 24 hours after injection of these compounds, PAS staining suggests that mucopolysaccharide metabolism may be affected (39). Thus, it seems possible that elevated testicular temperature may act directly or indirectly on the primary spermatocytes by interrupting their utilization or metabolism of carbohydrates, possibly glucose metabolism, and prevent further meiotic divisions.

Neither the sham operation nor exposure to abdominal temperature for 48 hours had any influence on the weight and the RNA and DNA content of the testes. These results suggest that nucleic acid synthesis and cell numbers remained relatively constant during the time the scrotal testes were exposed to the abdominal cavity. An alternative explanation for the failure to detect alterations in testicular nucleic acids is that the testes may be required to reside in the abdominal cavity for longer periods of time in order to demonstrate a measurable difference in testicular nucleic acid content.

Autoradiographic studies indicate that DNA synthesis normally occurs within the first quarter (stages IX through XIII) of the testicular phase of spermatogenesis (39). In the rat and mouse, DNA synthesis is confined to those cells of the spermatogenic series which are on or near the basement membrane and the "oldest" germinal cells showing DNA synthetic activity are the primary spermatocytes (39). Since the cell types classified as spermatogonia only comprise 1.7% of the total

testicular volume in the rat (87), a drastic reduction in DNA synthesis could occur among these cells but it could be masked by the remaining cell types which comprise 98.3% of the total DNA. Therefore, for a total DNA reduction to occur in the rat testis, it would be essential that a sufficient number of germinal cells be lost. The latter suggestion could only result from long term exposure to abdominal temperature. Since the total testicular DNA content remained relatively constant in the present study, it can be argued that complete distruction did not occur among the various germinal cell types as a result of exposing the scrotal testis to abdominal temperature for 48 hours.

The total testicular RNA was quantitated in an attempt to justify any parallel changes that may precede the changes in testicular soluble protein. Since there were no significant changes in total testicular RNA to be associated with soluble protein content, it must be kept in mind that there are as many types of RNA as there are different proteins. Thus, it seems possible that synthesis of small quantities of "essential" RNA molecules for enzyme or non-enzyme protein synthesis and their absence could not be distinguished by the RNA assay procedure used. LeVier (54) suggests that the hexomonophosphate shunt in rat testicular tissue has a 32°C temperature optimum. Hence, temperatures above 32°C would limit the availability of ribose which is necessary for nucleic acid synthesis. Therefore, it seems likely that long term exposure of scrotal testes to abdominal temperature reduces RNA synthesis because adequate supplies of pentose sugars may be limited.

The results of the present study revealed that the soluble protein content of the testes declined an average of 2.6 mg/g from control values within 48 hours after relocating the scrotal testes in the abdominal

cavity ($P<0.01$). These findings suggest that glucose entry into cells and its subsequent catabolism was not sufficient to support adequate high-energy supplies (ATP) which are essential for protein maintenance and biosynthetic processes. This interpretation of the present results is in agreement with the observation that testicular metabolism and biosynthetic processes are dependent on glucose as a primary source of energy (31,84). Protein biosynthesis *in vitro* in testicular tissue has been shown to be dependent upon exogenous glucose as a source of energy and glucose is known to stimulate protein biosynthesis (26,27,62). The stimulation of protein biosynthetic activity by glucose appears to be specific for the testis, since eleven other organs examined by Davis *et al.* (26) showed no increase or only a slight increase in incorporation of ^{14}C-amino acids into precipitated proteins. Means and Hall (62) attribute the increase in protein biosynthesis in testicular tissue to an adequate supply of adenosine triphosphate that can only be maintained in the presence of exogenous glucose in the incubation system. The fact that testicular protein synthesis seems to exist in an equilibrium between protein synthesis and degradation, forcing anaerobic metabolism on a normally aerobic tissue would upset this equilibrium and it could initiate degradation and hinder protein synthetic processes. Therefore, the observation of a significant ($P<0.01$) reduction in the soluble protein content of the testes after they had been exposed to the abdominal cavity for as long as 48 hours may be due to the fact that the available energy reserves had been exhausted. Presumably, exposing the testes to abdominal temperatures for time periods longer than 48 hours would result in a much larger reduction in protein synthesis since the ATP reserves should be completely exhausted. This assumption is supported by

the work of Means and Hall (62) since they observed a reduction in ATP levels to one-third that of normal within 30 minutes in the absence of glucose *in vitro*.

The rate of a chemical reaction doubles with each $10^{o}C$ rise in temperature (41). The increased demand for high energy compounds at elevated temperature must be met by supplying adequate substrates for producing this high energy. Failure to supply adequate substrates would disrupt cellular function and result in an interruption of metabolic processes. If the supply of substrate and oxygen for aerobic oxidation are both limiting, as may be the case for the testis (3,35), then an increase in temperature would create a greater demand for energy for maintaining cellular metabolism. Thus, glucose metabolism may occur either aerobically or anaerobically being influenced by limiting factors that regulate the rate of enzymatic reactions.

The failure to detect any significant changes in either the total or specific enzyme activity of pyruvate kinase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase suggests that these enzymes were not critical to testicular glucose metabolism and presumably were functional even after the temperature was elevated about $5^{o}C$. Relocating the testes from the scrotum to the abdomen had no deleterious effect on lactate dehydrogenase activity suggesting that the conversion of substrate (pyruvate) to product (lactate) was possible. The fact that rat testicular tissue metabolizes glucose anaerobically when it is exposed to body temperatures suggests that acetate metabolism via the citric acid cycle may be reduced so that lactic acid could accumulate without any measurable increase in lactate dehydrogenase activity (35). Since the activity of pyruvate kinase and lactate dehydrogenase was not

influenced by exposing the testes to abdominal temperature for 48 hours it seems logical to suggest that the concentrations of both of these enzymes were present to catalize their particular reactions. This would indicate that induction or repression of enzyme synthesis was not a factor and that the enzyme molecule had not lost its affinity for substrate or altered its structural conformation necessary for enzymatic activity.

The results of this study show that the early deleterious effects of abdominal temperature on testicular glucose metabolism significantly ($P<0.05$) reduced the total and specific activity of phosphofructokinase as well as the total activity of hexokinase. These two enzymes are the regulator enzymes in the glycolytic pathway. The initial increase in total hexokinase activity during the first 2 hours after relocating the testes in the abdominal cavity may reflect an increase in glucose metabolism due to the increase in testicular temperature. The continual decline in total hexokinase activity after the first 2 hours suggests that enzyme synthesis was inhibited or that its product, glucose-6-phosphate, inhibited hexokinase activity. The former interpretation must be considered since there were no indications that protein synthetic material (RNA) was reduced. The latter interpretation may be more plausible since an increase in the level of glucose-6-phosphate could cause a continual decline in phosphofructokinase activity, between 0 and 48 hours. Additional support for this speculation may be derived from the fact that the testis shifts from an aerobic to an anaerobic type of metabolism after being exposed to elevated temperature, so that glucose-6-phosphate could not be metabolized via the hexomonophosphate shunt. The gradual decline in total hexokinase activity was probably due to an inhibition by glucose-6-phosphate. Consequently, it is possible to

argue that glucose may not be trapped by the cells in the testes since an accumulation of glucose-6-phosphate would continue to depress hexokinase activity. Under these conditions, glucose supplies to the testis would become limiting within a short time (2-4 hours) after exposing a scrotal testis to the abdominal cavity.

In contrast to the increase in hexokinase activity, the total and specific activity of phosphofructokinase declined during the first 2 hours after exposure to abdominal temperature indicating that the activity of this regulatory enzyme was sensitive to abdominal temperature. The observed decline in the specific activity of phosphofructokinase by 48 hours suggests that the loss of activity of this enzyme occurs faster than the degradation of soluble protein. Thus, a reduction in the activity of the glycolytic pathway may be brought about by a reduction in phosphofructokinase activity followed by a reduction in the ability of hexokinase to trap glucose within the tissue some 8 hours later. The ability of glucose-6-phosphate to inhibit (product inhibition) hexokinase activity may not be alleviated at body temperature since the hexomonophosphate shunt has a 32^{o}C optimum in testicular tissue (54). These findings suggest that glycolysis was inhibited by a reduction in the activity of enzymes associated with regulation of glucose metabolism. Furthermore, the loss of activity of the glucose metabolizing enzymes involved in glycolysis occurred prior to detecting changes in either the soluble protein content or the morphology of the seminiferous tubule. Since elevated temperatures increase the rate of protein degradation in the testis, it is tempting to speculate that the synthesis or the structural configuration of enzyme protein associated with regulatory steps for glycolysis is temperature sensitive. Consequently, ATP levels may

not play a major role in regulating the regulatory activity of phosphofructokinase at abdominal temperature. This speculation is supported by the finding that the specific acitivity of phosphofructokinase (an ATP dependent enzyme) declined faster than the soluble protein content of the testis after exposure to abdominal temperature for 48 hours.

The author realizes that portions of this discussion may be highly speculative, nevertheless, the results presented here show a reduction in protein biosynthesis and the activities of phosphofructokinase and hexokinase following artificial cryptorchidism. These results suggest that inadequate energy supplies due to a reduction in glucose metabolism may be the primary factor in degeneration of primary spermatocytes following temperature elevation. Further studies should be aimed at measuring the actual products accumulated in association with enzyme activities in testicular tissue subjected to abdominal temperature in order to evaluate the flux of substrate through various metabolic pathways. Other studies could involve kinetic investigations and characterization of the two enzymes, hexokinase and phosphofructokinase, which seem to be drastically affected under the experimental conditions described here. Nevertheless, the findings presented in this study should serve as a basis to more adequately test hypothesis concerning the mechanisms by which hyperthermia causes degeneration of the spermatogenic and steroidogenic functions of the scrotal testis.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Experiments were designed to study the early effects of abdominal temperature on the activity of selected glucose metabolizing enzymes, the level of some biochemical constituents and the morphological integrity of testicular tissue obtained from 127 sexually mature male rats. The treatments consisted of either sham-operation or exposure to abdominal temperature for either 0, 2, 4, 8, 12, 24 or 48 hours.

The results of these experiments indicate that the sham operation had no significant influence on either the weight or the soluble protein content of the testes. Furthermore, neither the total nor the specific activity of the five glucose metabolizing enzymes was altered by the sham operation.

Relocating scrotal testes in the abdominal cavity for periods as long as 48 hours had no measurable affect on paired testis weight, testicular RNA and DNA levels and the total or specific activity of glucose-6-phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase. These findings indicate that not all of the criteria measured were affected by the elevated temperature after 48 hours exposure to abdominal temperature, and that cell numbers and affinity of some enzymes for their respective substrates were not drastically altered. Placing the testes in the abdominal cavity reduced the soluble protein content of the testes after they were exposed to the elevated

temperature of the abdomen for 48 hours ($P<0.01$). The total activity of phosphofructokinase and hexokinase was significantly ($P<0.05$) reduced within 4 and 12 hours, respectively. Hexokinase specific activity was not affected by abdominal temperature but phosphofructokinase specific activity dropped significantly ($P<0.05$) below control levels within 8 and 48 hours after exposing the testes to abdominal temperature. This latter effect suggests that glycolysis may not be functional at elevated temperatures due to the degradation of phosphofructokinase, a regulatory glycolytic enzyme. The ability of testicular tissue to trap glucose appeared to become limited some 8 hours after phosphofructokinase activity dropped because glucose levels may have been limited due to the inhibition of hexokinase by glucose-6-phosphate. The failure of glucose metabolism would in turn result in a reduction of such high energy compounds as ATP, ADP and these compounds are essential for the biosynthesis of both enzyme and non-enzyme proteins in rat testicular tissue. The absence of adequate energy supplies eventually modifies the resting primary spermatocytes since meiotic divisions appeared to be inhibited at stages IX to XIII of spermatogenesis.

These results indicate that abdominal temperature exhibits its deleterious effects on the metabolic pathways associated with testicular glucose metabolism about 38 to 44 hours prior to any noticeable histopathological changes.

SELECTED BIBLIOGRAPHY

1. Albert, A. In Sex and Internal Secretions. Ed. W. C. Young. Baltimore: Williams and Wilkens Company, 1961.

2. Asdell, S. A. and G. W. Salisbury. "The Rate at Which Spermatogenesis Occurs in the Rabbit." Anat. Rec. 80:145, 1941.

3. Baldwin, D. M. and L. L. Ewing. "An Enzymatic Comparison of Glucose Metabolism in the Rabbit and Chicken Testes and Kidney Cortex." Comp. Biochem. Physiol. 23:569, 1967.

4. Bloom, W. and D. W. Fawcett. A Textbook of Histology. Philadelphia: W. B. Saunders Company, 1962.

5. Bowler, K. "The Effects of Repeated Temperature Application to Testes on Fertility in Male Rats." J. Reprod. Fertil. 14: 171, 1968.

6. Bucher, T. and G. Pfleiderer. In Methods in Enzymology, Vol. 1. Eds. S. P. Colowick and N. O. Kaplan. New York: Academic Press, 1955, p. 435.

7. Burton, K. "A Study of the Conditions and Mechanisms of the Diphenylamine Reaction for the Colorimetric Estimation of Dioxyribonucleic Acid." Biochem. J. 62:315, 1956.

8. Buyer, R. and J. R. Davis. "Species Variation in the Effect of Temperature on the Incorporation of L-lysine-U-^{14}C Into Protein of Testis Slices." Comp. Biochem. Physiol. 17:151, 1966.

9. Ceriotti, G. J. "Determination of Nucleic Acids in Animal Tissues." Biol. Chem. 214:59, 1955.

10. Chowdhurg, A. K. and E. Steinberger. "A Quantitative Study of the Effect of Heat on Germinal Epithelium of Rat Testes." Am. J. Anat. 115:509, 1964.

11. Clegg, E. J. "Some Effects of Artificial Cryptorchidism on the Accessory Reproductive Organs of the Rat." J. Endocrinol. 20:210, 1960.

12. Clegg, E. J. "Further Studies on Artificial Cryptorchidism: Quantitative Changes in the Sertoli Cells of the Rat Testis." J. Endocrinol. 21:433, 1961.

13. Clegg, E. J. "Studies on Artificial Cryptorchidism: Morphological and Quantitative Changes in the Sertoli Cells of the Rat Testes." J. Endocrinol. 26:567, 1963.

14. Clegg, E. J. "Studies on Artificial Cryptorchidism: Degenerative and Regenerative Changes in the Germinal Epithelium of the Rat Testis." J. Endocrinol. 27:241, 1963.

15. Clegg, E. J. "Studies on Artificial Cryptorchidism: Compensatory Changes in the Scrotal Testes of Unilaterally Cryptorchid Rats." J. Endocrinol. 33:259, 1965.

16. Clermont, Y. and S. C. Harvey. "Duration of the Cycle of the Seminiferous Epithelium of Normal, Hypophysectomized and Hypophysectomized-hormone Treated Albino Rats." Endocrinol. 76:80, 1965.

17. Clermont, Y. and C. P. Leblond. "Renewal of Spermatogonia in the Rat." Am. J. Anat. 93:475, 1953.

18. Clermont, Y. and C. P. Leblond. "Differentiation and Renewal of Spermatogonia in the Monkey, Macacus rhesus." Am. J. Anat. 104:237, 1959.

19. Clermont, Y. and B. Perey. "The Stages of the Cycle of the Seminiferous Epithelium of the Rat: Practical Definitions in PA-Schiff-hematoxylin and Hematoxylin-eosin Stained Sections." Rev. Canad. Biol. 16:451, 1957.

20. Crew, F. A. E. "A Suggestion as to the Cause of the Aspermatic Condition of the Imperfectly Descended Testis." J. Anat. 56:98, 1922.

21. Cross, B. A. and L. A. Silver. "Neurovascular Control of Oxygen Tension in the Testis and Epididymis." J. Reprod. Fertil. 3:377, 1962.

22. Davis, J. R., E. B. Baietto, and S. D. Delisi. "A Comparison of the Aerobic and Anaerobic Incorporation of L-lysine-U-^{14}C into Protein of Various Tissues of the Rat." Comp. Biochem. Physiol. 17:697, 1966.

23. Davis, J. R. and C. F. Firlit. "Effect of Glucose on Uptake of L-lysine-H^3 in Cells of the Seminiferous Epithelium." Am. J. Physiol. 209:425, 1965.

24. Davis, J. R. and C. F. Firlit. "The Germinal Epithelium of Cryptorchid Testes Experimentally Induced in Prepuberal and Adult Rats." Fertil. Steril. 17:187, 1966.

25. Davis, J. P., C. F. Firlit, and M. A Hollinger. "Effect of Temperature on Incorporation of L-lysine-U-^{14}C Into Testicular Proteins." Am. J. Physiol. 204:696, 1963.

26. Davis, J. R. and R. N. Morris. "Effect of Glucose on Incorporation of L-lysine-U^{14}C Into Testicular Proteins." Am. J. Physiol. 205:833, 1963.

27. David, J. R., R. N. Morris, and M. A. Hollinger. "Incorporation of L-lysine-U-^{14}C Into Proteins of Cryptorchid Testis Slices." Am. J. Physiol. 207:50, 1964.

28. Davis, J. R., R. N. Morris, and M. A. Hollinger. "Comparison of Testicular Protein Labeling in Cryptorchidism Induced in Prepubertal and Adult Rats." J. Reprod. Fertil. 10:149, 1965.

29. Dickens, F. and G. D. Greville. "Metabolism of Normal and Tumor Tissue. VIII. Respiration in Fructose and in Sugar Free Media." Biochem J. 27:832, 1933.

30. Dickens, F. and G. D. Greville. "Metabolism of Normal and Tumor Tissue. IX. Ammonia and Urea Formation." Biochem. J. 27:1123, 1933.

31. Elliott, K. A. C., M. E. Creig, and M. P. Benoy. "The Metabolism of Lactic and Pyruvic Acids in Normal and Tumor Tissues. III. Rat Liver, Brain and Testis." Biochem. J. 31:1003, 1937.

32. Ewing, L. L., E. R. Baird, and N. L. VanDemark. "Comparative Metabolic Activity of Testis and Kidney Cortex Slices of Normal and Hypoglycemic Chickens and Rabbits." Comp. Biochem. Physiol. 17:455, 1966.

33. Ewing, L. L. and N. L. VanDemark. "The Effect of Body and Scrotal Temperature on Metabolic Activity of Rabbit Testis in vitro." J. An. Sci. 19:1320, 1960.

34. Ewing, L. L. and N. L. VanDemark. "Metabolic Activity of Rabbit Testicular Tissue After Various Periods in the Abdomen." J. Dairy Sci. 44:1188, 1961.

35. Ewing, L. L. and N. L. VanDemark. "Factors Affecting Testicular Metabolism and Function. II. Effect of Temperature Elevation in vivo on subsequent Metabolic Activity of Rabbit Testicular Tissue in vitro." J. Reprod. Fertil. 6:9, 1963.

36. Ewing, L. L. and N. L. VanDemark. "Factors Affecting Testicular Metabolism and Function. III. Effect of in vivo Temperature Elevation on Tissue Slices and Perfused Testes in the Rabbit." J. Reprod. Fertil. 6:17, 1963.

37. Firlit, C. F. and J. R. Davis. "Radio-autographic Incorporation of L-lysine-^{3}H Into Protein of Cells of the Germinal Epithelium in Cryptorchidism." J. Reprod. Fertil. 11:125, 1966.

38. Fleeger, J. L. and N. L. VanDemark. "Effect of CO_2 Level and Temperature on Testicular Tissue Metabolism." Fed. Proc. 24:700, 1965.

39. Fox, B. W. and M. Fox. "Biochemical Aspects of the Action of Drugs on Spermatogenesis." Pharm. Rev. 19:21, 1967.

40. Free, M. J. and N. L. VanDemark. "Gas Tension in Spermatic and Peripheral Blood of Rams With Normal and Heat-Treated Testis." Am. J. Physiol. 214:863, 1968.

41. Giese, Arthur C. Cell Physiology, Third Edition. Philadelphia: W. B. Saunders Company, 1968, p. 235.

42. Glock, G. and P. McLean. "Further Studies on the Properties and Assay of Glucose-6-Phosphate Dehydrogenase and 6-phosphogluconate Dehydrogenase of Rat Liver, I." Biol. Chem. 55:400, 1953.

43. Glover, T. D. "The Effect of Scrotal Insulation and the Influence of the Breeding Season Upon Fructose Concentration in the Semen of the Ram." J. Endocrinol. 13:235, 1956.

44. Gomori, B. "Preparation of Buffers for use in Enzyme Studies." In Methods in Enzymology. Eds. S. P. Colowick and N. O. Kaplan. New York: Academic Press, 1955, p. 138.

45. Greep, R. O. Histology, Second Edition. New York: McGraw-Hill Book Company, 1966, p. 701.

46. Hannon, J. P. and D. A. Vaughan. "Effect of Prolonged Cold Exposure on the Glycolytic Enzymes of Liver and Muscle." Am. J. Physiol. 198:375, 1960.

47. Harvey, S. C. "Effect of Hormones on Spermatogenesis in the Rat." (unpub. Ph.D. thesis, McGill University, Montreal, Canada, 1966).

48. Hollinger, M. A. and J. R. Davis. "Metabolic Fate of D-glucose-U-C^{14} in Slices of Scrotal and Cryptorchid Testes." Fed. Proc. 25:313, 1966.

49. Idanpaan-Heikkila, P. "Effect of Local Heat in vitro on the Fine Structure of Basement Membrane and the Sertoli Cells of the Rat Testis." Fertil. Steril. 17:689, 1966.

50. Kornberg, A. In Methods in Enzymology, Vol. 1. Eds. S. P. Colowick and N. O. Kaplan. New York: Academic Press, 1955, p. 441.

51. Kornberg, A. and B. L. Horecker. In Methods in Enzymology, Vol. 1. Eds. S. P. Colowick and N. O. Kaplan. New York: Academic Press, 1955, p. 323.

52. Lardy, H. A. and R. E. Parks. In Enzymes. Ed. O. H. Gaebler. New York: Academic Press, 1956.

53. Leblond, C. P. and Y. Clermont. "Definition of the Stages of the Cycle of the Seminiferous Epithelium in the Rat." Ann. N. Y. Acad. Sci. 55:548, 1952.

54. Le Vier, R. R. "In vitro Temperature Effects of Testicular Metabolism in the Rat." (unpub. Ph.D. thesis (abstract), Iowa City, Iowa, 1968).

55. Ling, K., W. Bryrne, and H. Lardy. In Methods in Enzymology, Vol. 1. Eds. S. P. Colowick and N. O. Kaplan. New York: Academic Press, 1955, p. 306.

56. Lowry, O. H. and J. V. Passonneau. "The Relationships Between Substrates and Enzymes of Glycolysis in Brain." J. Biol. Chem. 239:31, 1964.

57. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. "Protein Measurement With Folin Phenol Reagent." J. Biol. Chem. 193:265, 1951.

58. Mancini, R. E., J. C. Penhos, I. A. Izquerdo, and J. J. Heinrich. "Effect of Acute Hypoglycemia on Rat Testis." Proc. Soc. Exp. Biol. Med. 104:699, 1960.

59. Mansour, T. E. "Studies on Heart Phosphofructokinase: Purification, Inhibition and Activation." J. Biol. Chem. 238:2285, 1963.

60. Mansour, T. E. "Factors Influencing Activation of Phosphofructokinase." Pharm. Rev. 18:173, 1966.

61. Means, A. R. and P. F. Hall. "Protein Biosynthesis in the Testes. I. Comparison Between Stimulation by FSH and Glucose." Endocrinol. 82:597, 1968.

62. Means, A. R. and P. F. Hall. "Protein Biosynthesis in the Testes. II. Role of Adenosine Triphosphate (ATP) in Stimulation by Glucose." Endocrinol. 83:86, 1968.

63. Moore, C. R. "Cryptorchidism Experimentally Produced." Anat. Rec. 24:383, 1922.

64. Moore, C. R. "On the Relationship of the Germinal Epithelium to the Position of the Testis." Anat. Rec. 25:142, 1923.

65. Moore, C. R. "Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. VI. Testicular Reactions in Experimental Cryptorchidism." Amer. J. Anat. 34:269, 1924.

66. Moore, C. R. "Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. VIII. Heat Application and Testicular Degeneration on the Function of the Scrotum." Amer. J. Anat. 34:337, 1924.

67. Moore, C. R. "The Behavior of the Germinal Epithelium in Testis Grafts and in Experimental Cryptorchid Testes." Science. 59:41, 1924.

68. Moore, C. R. "Experimental Studies on the Male Reproductive System." J. Urology 65:497, 1951.

69. Moore, C. R. and H. D. Chase. "Heat Application and Testicular Degeneration." Anat. Rec. 26:344, 1923.

70. Moore, C. R. and R. Oslund. "Experimental Studies on Sheep Testes." Anat. Rec. 26:343, 1923.

71. Moore, C. R. and R. Oslund. "Experiments on the Sheep Testis: Cryptorchism, Vasectomy and Scrotal Insulation." Am. J. Physiol. 67:595, 1924.

72. Moore, C. R. and W. J. Quick. "A Comparison of Scrotal and Peritoneal Temperatures." Anat. Rec. 26:344, 1923.

73. Moore, C. R. and W. J. Quick. "The Scrotum as a Temperature Regulator for the Testes." Am. J. Physiol. 68:70, 1924.

74. Morris, R. N. and J. R. Davis. "Glucose Protection Against Heat Induced Inhibition of Rat Testicular Protein." Fed. Proc. 25:313, 1966.

75. Moule, G. R. and G. M. H. Waites. "Seminal Degeneration in the Ram and its Relation to the Temperature of the Scrotum." J. Reprod. Fertil. 5:433, 1963.

76. Nelson, W. O. "Mammalian Spermatogenesis: Effect of Experimental Cryptorchidism in the Rat and Non-descent of the Testis in Man." Rec. Prog. Horm. Res. 6:29, 1951.

77. Nelson, W. O. and C. G. Heller. In Recent Progress in Hormone Research, Vol. III. Ed. G. Pincus. New York: Academic Press, 1948.

78. Nelson, W. O. and E. Steinberger. "Effects of Nitrofuran Compounds on the Testis of the Rat." Fed. Proc. 12:103, 1953.

79. Newsholme, E. A. and P. J. Randle. "Regulation of Glucose Uptake by Muscle: Effects of Anoxia, Insulin, Adrenaline and Prolonged Starving on Concentration of Hexose Phosphates in Isolated Rat Diaphragm and Perfused Isolated Rat Heart." Biochem. J. 80:655, 1961.

80. Niemi, M. and M. Kormano. "Response of the Cycle of the Seminiferous Epithelium of the Rat Testes to Artificial Cryptorchidism." Fertil. Steril. 16:236, 1965.

81. Oakberg, E. F. "A Description of Spermatogenesis in the Mouse and its use in the Analysis of the Cycle of the Seminiferous Epithelium and Germ Cell Renewal." Am. J. Anat. 99:391, 1956.

82. Ortavant, R. In Reproduction in Domestic Animals, Vol. II. Eds. H. H. Cole and P. T. Cupps. New York: Academic Press, 1959.

83. Passonneau, J. V. and O. H. Lowry. "Phosphofructokinase and the Pasteur Effect." Biochem. Biophys. Res. 7:10, 1962.

84. Paul, M. F., H. E. Paul, F. Kopko, M. Bryson, and C. Harrington. "Carbohydrate Metabolism Studies on the Testis of Rats fed Certain Nitrofurans." Endocrinol. 53:585, 1953.

85. Paul, M. F., H. E. Paul, F. Kopko, M. Bryson, and C. Harrington. "Inhibition by Furacin of Citrate Formation in Testis Preparations." J. Biol. Chem. 206:490, 1954.

86. Regen, D. M., W. W. Davis, H. E. Morgan, and C. R. Park. "The Regulation of Hexokinase and Phosphofructokinase Activity in Heart Muscle: Effects of Alloxan Diabetes, Growth Hormone, Cortisol and Anoxia." J. Biol. Chem. 239:43, 1964.

87. Roosen-Runge, E. C. "Quantitative Investigations on Human Testicular Biopsies. I. Normal Testis." Fertil. Steril. 7:251, 1956.

88. Rose, I. A. and E. L. O'Connell. "The Role of Glucose-6-Phosphate in the Regulation of Glucose Metabolism in Human Erythrocytes." J. Biol. Chem. 239:12, 1964.

89. Rose, I. A., J. V. B. Warms, and E. L. O'Connell. "Role of Inorganic Phosphate in Stimulating the Glucose Utilization of Human Red Blood Cells." Biochem. Biophys. Res. Comm. 15:33, 1964.

90. Schneider, W. C. "Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Deoxypentose Nucleic Acid and of Pentose Nucleic Acid." J. Biol. Chem. 161:293, 1945.

91. Shetteles, L. B. "Effects of low Oxygen Tension on Fertility in Adult Male Guinea Pigs." Fed. Proc. 6:200, 1947.

92. Smith, P. E. "Comparative Effects of Hypophysectomy and Therapy on the Testis of Monkeys and Rats." In Les Hormones Sexuelles. Ed. L. Bronha. Paris: Foundation Singer-Polignac Collogium, Vol. 3, 1938.

93. Smith, P. E. In Sex and Internal Secretions, Second Edition. Eds. E. Allen, C. H. Danforth and E. A. Daisy. Baltimore: The Williams and Wilkins Company, 1939.

94. Sripati, C. E. "On the Prevention of Loss of RNA Into Lipid Solvents." Experientia 23:695, 1967.

95. Steel, R. G. D. and J. H. Torrie. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company, 1960.

96. Turner, C. D. General Endocrinology, Fourth Edition. Philadelphia: W. B. Saunders Company, 1966.

97. Ui, M. "A Role of Phosphofructokinase in pH-dependent Regulation of Glycolysis." Biochem. Biophys. Acta. 124:310, 1966.

98. Waites, G. M. H. and G. R. Moule. "Regulation of Vascular Heat Exchange to Temperature Regulation in the Testis of the Ram." J. Reprod. Fertil. 2:213, 1961.

99. Waites, D. G. and B. P. Setchell. "Effect of Local Heating on Blood Flow and Metabolism in the Testis of the Conscious Ram." J. Reprod. Fertil. 8:339, 1964.

100. Wu, R. "Control of Glycolysis by Phosphofructokinase in Slices of Rat Liver. Novikoff Hepatoma a Adeno Carcinomas." Biochem. Biophys. Res. Comm. 14:79, 1963.

101. Young, W. C. "The Influence of High Temperature on the Guinea Pig Testis Histological Changes and Effects on Reproduction." J. Exp. Zool. 49:459, 1927.

APPENDIX A

TABLE V

CHEMICALS

Chemicals purchased from the Sigma Chemical Company, St. Louis, Missouri:

Adenosine-5'-diphosphate, sodium salt, Grade 1

Adenosine-5'-triphosphate, disodium salt, Sigma Grade

D-cysteine hydrochloride

Dihydronicotinamide adenine dinucleotide phosphate, reduced form, Disodium, Grade III

Fructose-6-phosphate, sodium salt, Grade 1

Glucose-6-phosphate, disodium salt, Sigma Grade

Glucose-6-phosphate, dehydrogenase, from yeast, Type V

Lactic acid dehydrogenase, from beef heart, Type III

Nicotinamide adenine dinucleotide phosphate, reduced form, sodium salt, Sigma Grade

Phospho(enol)pyruvate, trisodium salt

Pyruvate, sodium salt, Type II

Triethanolamine hydrochloride

Tris (hydroxymethyl) aminomethane, Sigma 7-9

Chemicals purchased from Calbiochem, Los Angeles, California:

Aldolase, rabbit muscle, A Grade

α-Glycerophosphate dehydrogenase, rabbit muscle, A Grade

APPENDIX B

TABLE VI

PROCEDURE FOR PREPARATION OF REAGENTS USED

Buffers

Tris (hydroxymethyl) aminomethane - (mol wt = 121.1)
0.1 M (Tris) Buffer = 1.211 g/100 ml DDW*
adjusted to pH 7.0, 8.0 or 9.0

Triethanolamine-hydrochloride - (mol wt = 185.6)
0.1 M Tri-HCl Buffer = 1.856 g/100 ml
DDW adjusted to pH 8.0

Phosphate Buffer pH 7.4 was prepared according to the method described by Gomori (44).

Metal Solutions

Magnesium Chloride		(mol wt = 95.23)
0.75 M $MgCl_2$	=	7.132 g/100 ml DDW
0.10 M $MgCl_2$	=	0.952 g/100 ml DDW
0.01 M $MgCl_2$	=	95.23 g/100 ml DDW
Potassium Chloride		(mol wt = 74.55)
0.75 M KCl	=	5.591 g/100 ml DDW
0.154 M KCl	=	11.48 g/liter DDW

Dilution of Coupling Enzymes

Aldolase (A Grade) 168 E.U./ml $(NH_4)_2SO_4$
1:50 dilution = 3.6 E.U./ml Tris Buffer pH 7.0

α-Glycerophosphate Dehydrogenase 50 E.U./ml $(NH_4)_2SO_4$
1:50 dilution = 1.0 E.U./ml Tris Buffer pH 7.0

Lactate Dehydrogenase (Type III) 10 mg Protein/ml $(NH_4)_2SO_4$
1:100 dilution = 0.1 mg protein/ml Tris Buffer pH 7.0

Glucose-6-phosphate Dehydrogenase (Type V) 50 E.U./0.1 ml $(NH_4)_2SO_4$
10 units/ml = 0.02 ml G-6-PDH/ml Tris Buffer pH 7.0

TABLE VI (Continued)

Cofactors and Substrates

D-Cysteine-hydrochloride (for wt = 175.6)
0.2 M = 351.2 mg/10 ml Tris buffer pH 7.0

Adenosine triphosphate (for wt = 629.432)
.05 M = 314.7 mg/10 ml Tris buffer pH 7.0

Adenosine diphosphate (for wt = 504.6)
0.05 M = 252.3 mg/10 ml DDW

Fructose-6-phosphate (for wt = 385.66)
0.1 M = 385.7 mg/10 ml DDW

Glucose (mol wt = 180.16)
0.1 M = 180.16 mg/10 ml DDW

Glucose-6-phosphate (for wt = 325.3
0.05 M = 162.2 mg/10 ml DDW

NADH (for wt = 778.26)
0.002 M = 15.57 mg/10 ml DDW

NADP (for wt = 862.1)
0.003 M = 25.0 mg/10 ml DDW

Phospho(enol)pyruvate (for wt = 347.1)
0.1 M = 347.1 mg/10 ml DDW

Pyruvate (mol wt = 110.0
0.01 M = 11.0 mg/10 ml DDW

---

*DDW = Double distilled water (glass)

APPENDIX C

TABLE VII

ANALYSIS OF VARIANCE OF PAIRED TESTIS WEIGHTS COMPARING REPLICATIONS AND TREATMENTS AFTER RELOCATING RAT TESTES IN ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	41	2.238	----	----
Replicates	5	0.998	0.1996	7.103**
Treatment	6	0.396	0.066	2.349
Error	30	0.844	0.0281	----

**($P<0.01$).

TABLE VIII

ANALYSIS OF VARIANCE OF THE SOLUBLE PROTEIN CONTENT COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATING THE TESTES TO ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	104	948.45	----	----
Replicates	14	371.49	26.535	4.606**
Treatment	6	93.02	15.503	2.69*
Error	84	483.94	5.761	----

*($P<0.05$).

**($P<0.01$).

TABLE IX

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO THE MEAN PROTEIN CONTENT OF TESTES AFTER EXPOSURE TO ABDOMINAL CAVITY FOR DIFFERENT TIME PERIODS

Treatment (hours)	48	4	24	8	12	2	0
Mean	26.87	27.61	27.70	28.31	29.04	29.47	29.52
Value of p (d.f. = 84)	2	3	1% 4	5	6	7	
SSR	3.71	3.86	3.98	4.06	4.11	4.17	
LSR	2.30	2.39	2.47	2.52	2.55	2.58	
($S_{\bar{x}}$ = .62)							

[1]Steel and Torrie (95).

TABLE X

ANALYSIS OF VARIANCE OF RIBONUCLEIC ACID CONTENT COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	97	7.05	----	----
Replicates	13	2.924	0.2249	4.516**
Treatments	6	0.240	0.0400	0.803
Error	78	3.886	0.0498	----

**($P<0.01$).

TABLE XI

ANALYSIS OF VARIANCE OF DEOXYRIBONUCLEIC ACID CONTENT COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	97	3.135	----	----
Replicates	13	0.776	0.0597	2.236**
Treatments	6	0.274	0.0457	1.712
Error	78	2.085	0.0267	----

**(P<0.01).

TABLE XII

ANALYSIS OF VARIANCE OF HEXOKINASE TOTAL ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	48	3.505	----	----
Replicates	6	2.650	0.442	32.028**
Treatment	6	0.360	0.060	4.350**
Error	36	0.495	0.0138	----

**(P<0.01).

TABLE XIII

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED OT THE MEAN TOTAL ACTIVITY OF HEXOKINASE AFTER EXPOSURE OF THE TESTES TO THE ABDOMINAL CAVITY FOR DIFFERENT TIME PERIODS

Treatment (hours)	48	24	12	0	8	4	2
Mean	1.39	1.45	1.49	1.57	1.58	1.60	1.67
Value of p (d.f. = 36)			5%				
	2	3	4	5	6	7	
SSR	2.86	3.01	3.10	3.17	3.22	3.27	
LSR	.126	.132	.136	.139	.142	.144	
($S_{\bar{x}}$ = .044)							

[1]Steel and Torrie (95).

TABLE XIV

ANALYSIS OF VARIANCE OF PHOSPHOFRUCTOKINASE TOTAL ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	41	0.298	----	----
Replicates	5	0.173	0.0346	15.309**
Treatments	6	0.057	0.0095	4.204**
Error	30	0.068	0.00226	----

**(P<0.01).

TABLE XV

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO THE MEAN TOTAL ACTIVITY OF PHOSPHOFRUCTOKINASE AFTER EXPOSURE OF THE TESTES TO THE ABDOMINAL CAVITY FOR DIFFERENT TIME PERIODS

Treatment (hours)	48	24	8	12	4	2	0
Mean	.621	.635	.643	.661	.669	.702	.734
Value of p (d.f. = 30)	2	3	4	1% 5	6	7	
SSR	2.89	3.04	3.12	3.20	3.25	3.29	
LSR	.058	.061	.062	.064	.065	.066	
($S_{\bar{x}}$ = .020)							

[1]Steel and Torrie (95).

TABLE XVI

ANALYSIS OF VARIANCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE TOTAL ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	48	0.254	-----	-----
Replicates	6	0.101	0.017	3.985**
Treatment	6	0.030	0.005	1.466
Error	36	0.123	0.00341	-----

**(P<0.01).

TABLE XVII

ANALYSIS OF VARIANCE OF PYRUVATE KINASE TOTAL ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	34	36.36	----	----
Replicates	4	8.429	2.107	1.991
Treatment	6	6.762	1.127	1.065
Error	20	21.169	1.058	----

TABLE XVIII

ANALYSIS OF VARIANCE OF LACTATE DEHYDROGENASE TOTAL ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	41	533.426	----	----
Replicates	5	333.316	66.663	13.736**
Treatment	6	54.516	9.086	1.872
Error	30	145.594	4.853	----

**($P<0.01$).

TABLE XIX

ANALYSIS OF VARIANCE OF PHOSPHOFRUCTOKINASE SPECIFIC ACTIVITY COMPARING REPLICATIONS AND TREATMENTS AFTER TRANSLOCATION OF THE TESTES TO THE ABDOMINAL CAVITY

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F ratio
Total	41	5.058	----	----
Replicates	5	2.86	.572	13.62**
Treatment	6	0.95	.158	3.798**
Error	30	1.25	.042	----

**(P<0.01).

TABLE XX

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO THE MEAN SPECIFIC ACTIVITY OF PHOSPHOFRUCTOKINASE AFTER EXPOSURE OF THE TESTES TO THE ABDOMINAL CAVITY FOR DIFFERENT TIME PERIODS

Treatment (hours)	48	8	12	24	4	2	0
Mean	2.227	2.227	2.300	2.348	2.402	2.482	2.512

Value of p (d.f. = 30)	5%					
	2	3	4	5	6	7
SSR	.240	.253	.260	.266	.270	.274
LSR	2.89	3.05	3.13	3.20	3.25	3.30
($S_{\bar{x}}$ = .083)						

[1]Steel and Torrie (95).

[2]Mean x 10^{-2}.

VITA

Larry Max Schanbacher

Candidate for the Degree of

Master of Science

Thesis: EARLY EFFECTS OF ABDOMINAL TEMPERATURE ON RAT TESTICULAR TISSUE: A HISTOLOGICAL, BIOCHEMICAL AND ENZYMATIC STUDY

Major Field: Physiology

Biographical:

Personal Data: Born in Cherokee, Oklahoma, September 27, 1944, the son of Delbert W. and Glendola M. Schanbacher.

Education: Attended the public schools of Cherokee, Oklahoma; graduated from Cherokee High School, Cherokee, Oklahoma in May, 1962; received the Bachelor of Science degree from Northwestern State College, Alva, Oklahoma, with majors in Biology and Chemistry in August, 1966; completed the requirements for the Master of Science degree in May, 1969.

Professional Experience: Served as a laboratory assistant, Department of Biology, Northwestern State College, Alva, Oklahoma, September, 1965 to January, 1966; served as a Graduate Teaching Assistant, September, 1967 to January, 1968 and employed as a NASA Predoctoral Fellow, September, 1966 to August, 1969, in the Department of Physiology and Pharmacology at Oklahoma State University, Stillwater, Oklahoma.