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A Comparative Study of the Effect of Temperature on Testicular Protein Labeling in Common Laboratory Animals

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A COMPARATIVE STUDY OF THE EFFECT OF TEMPERATURE ON TESTICULAR PROTEIN LABELING IN COMMON LABORATORY ANIMALS

by

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LIFE

Richard Buyer was born in Chicago, Illinois on September 14, 1939. He attended both Proviso Township High School in Maywood, Illinois and Elmwood Park Community High School in Elmwood Park, Illinois. In June, 1961 he received the Degree of Bachelor of Science in Zoology from the University of Illinois at Urbana.

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In December, 1964 he was married to the former Miss Andrea Kay Radlove of Chicago, Illinois.

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CHAPTER I

INTRODUCTION

A. THE COMPARATIVE APPROACH

The comparative approach in biology (62) has served a dual purpose: first, extrapolation from one group of organisms to another can lead to biological generalizations; secondly, different kinds of organisms solve similar life problems in different ways. The first of these purposes emphasizes a basic similarity between organisms, whereas the second emphasizes differences.

In recent years much work has been done on cellular and molecular functions. Yet comparative physiology and comparative biochemistry do not restrict themselves to the description of life processes in terms of molecular mechanisms. All levels of organization are studied. As a result, the comparative scientist attempts to find biological meaning at the levels of cells, organs, species, and higher taxonomic categories. The comparative approach considers the spectrum within a given type of tissue, it considers the diversity of a function in different environments such as high or low temperatures, and it considers functional phylogenetic relationships. Above all it considers the integrated functions of homeostasis.

In any comparative study, two major factors are seen to play a predominant role in distinguishing between individuals or species. These factors are inheritance and environment. The study of genetic separation as well as the adaptive traits which uniquely suit the species to its ecological level enable the comparative scientist to classify a given species in terms of survival, reproduction and development, and aspects of its in-

internal state or its metabolic pattern.

B. THE TESTIS

During the course of development of the majority of mammals the testes leave their primitive lodgment in the body cavity and migrate posteriorly and ventrally to the terminal periphery where they protrude at the surface of the body wall. According to Crew (11) this protrusion constitutes the scrotum which varies in character from that of a pair of small ill-defined slightly elevated areas to that of a capacious, definite, pedunculated sac.

1. Phylogenetic Position

The phenomenon whereby the testes migrate to an extra-abdominal position occurs only in mammals; yet this process is subject to much variation. In some species it does not take place at all, while in others the ultimate position assumed by the descended organ may be either inguinal, perineal, or scrotal. In a third group of mammals, the testis is abdominal during the greater part of the year, but descends into the scrotum during the breeding season. This constitutes true periodic descent and species exhibiting it should be distinguished from others in which the testes, although normally scrotal or perineal, may at moments of fright and excitement be withdrawn, through the action of the cremaster muscle towards the peritoneal cavity.

2. Comparative Location of the Testis

The following is a brief summary of the location of the testes of six mammals: the adult rabbit, rat, mouse, golden hamster, guinea pig, and dog, according to the description of Eckstein and Zuckerman (19).

a. Rabbit

The testes of the rabbit are elongated or fusiform in shape. They lie in subcutaneous scrotal sacs which communicate freely with the peritoneal cavity, and can be reflexly withdrawn or moved through the inguinal canals into the abdomen.

b. Rat

The testes of the mature rat lie in a prominent subcutaneous swelling known as the scrotum. There are two separate scrotal sacs, each of which communicates with the peritoneal cavity through a wide inguinal canal; this permits the withdrawal of the gonads into the abdomen throughout life.

c. Mouse

Like the rat, the testes of the mouse form two connecting subcutaneous swellings. There is also free communication with the peritoneal cavity.

d. Hamster

Like both the rat and the mouse, the testes of the golden hamster are located in subcutaneous scrotal pouches which communicate freely with the peritoneal cavity.

e. Guinea Pig

The testes of the guinea pig are ovoid in shape and lie in separate pouches on either side of the penis, slightly below the level of the anus. There is a well-developed cremaster muscle which permits the withdrawal of the testes into the abdomen.

f. Dog

The scrotum is globular in shape and is situated between inguinal region and the anus. It is a pouch composed of skin and the dartos tunic. The

skin is well supplied with sebaceous follicles. The testes of the dog are comparatively small and usually arranged one behind the other in separate scrotal compartments.

Other investigators such as Wislocki (79) (19) have separated the position of the testes in mammals into two distinct groups: those which lie permanently in the abdomen and those which lie outside the abdominal cavity. Included in this latter category are the testes of the rat, mouse, hamster, rabbit, guinea pig, and dog. The testes of the first five mammals just mentioned are considered subintegumental (inguinal or perineal); thus, even though they are generally considered to be in a "scrotal position", nevertheless, they can still be voluntarily or reflexly withdrawn into the abdomen. In contrast, the testes of the dog are considered permanently scrotal as are those of most primates such as the monkey and man.

Moore (49) also makes this differentiation. He states that the guinea pig, rabbit, rat, and some other mammals differ from others in that the inguinal canal serves as a wide-open connection throughout life between the abdominal cavity and the cavity of the scrotum. The testes may be retracted into the abdominal cavity, but due to the connection existing between the epididymis and the bottom of the scrotal sac, they generally redescend into the scrotum on relaxation of the cremaster muscle.

According to Crew (11), the major difference between the scrotum and the abdominal cavity is that the temperature within the tunica vaginalis is appreciably lower than that within the general abdominal cavity. The scrotum is so constructed that it is exceedingly well-equipped with a temperature-regulating mechanism. It is known that the final stages of

spermatogenesis occur at an optimum temperature which is found within the scrotum and not that found within the abdominal cavity.

Yet this also presents variations. Some mammals such as the whale and elephant lack a scrotum, and the testes are continuously abdominal. Obviously, body temperature does not damage spermatogenesis in these forms (58). In other animals which do possess a scrotum, the testes occupy scrotal positions only during the breeding season. At the other times the testes are elevated into the abdominal cavity. However, even these animals appear to suffer no permanent damage during these periods of abdominal retention.

3. Effect of High Temperature on Testicular Morphology

It is generally believed that high environmental temperatures are deleterious to physiological systems (72), and as a result it is thought that high temperature could affect reproduction indirectly through such factors as stress and general well-being of the animal and also directly by influencing the formation of sperm or ova. Ulberg (74) states that an increase in environmental temperature above a certain point will cause an increase in body temperature, and this increase in body temperature has been seen to affect the fertility and sperm production of rams or bulls. Small differences in temperature have a great effect on the production and life of the spermatozoa in mammals. It has been suggested that the cause for sterility in abdominal testes is the higher temperature that exists in the abdomen than in the scrotum.

Many investigators have shown a degeneration of the germinal epithelium of the testis as a result of exposures to temperatures higher than those normally found in the scrotum. Moore and Chase (50) found that single applications of heat approximately 7° C. above body temperature have proved

capable of causing testicular degeneration. This type of degeneration was similar to that which Moore found in the testis of guinea pigs made artificially cryptorchid through elevation from the scrotum into the abdomen (51). By means of this work, Moore and Oslund (52) stated that the mammalian scrotum is a local thermoregulator for the testis. He noticed that if this function was prevented by external insulation against heat loss, the testis undergoes degeneration while still in the scrotum. In this way the animal is sterilized by its own body heat.

Young (81) showed that exposure of the scrotum of the guinea pig to water at 46°C. for 30 minutes and 47.5°C. for 15 minutes resulted in an almost complete degeneration and desquamation of the germinal epithelium of the testis. More evidence of the susceptibility of the testis to heat was provided by Fukui (25) who found that application of heat to the testes of rabbits at a temperature equivalent to the normal body temperature of the rabbit (40°C) resulted in defective spermatogenesis. Fukui was also able to produce these "heat testicles" in dogs, guinea pigs, and rats. He also showed that bathing the scrotum in hot water at 48°C. destroyed the spermatogenic epithelium of rabbits within one hour. One explanation for this was put forth by Glover and Young (26) who said that physiologic mechanisms, such as enzyme systems, are likely to be impaired by temperatures approaching 50°C.

In contrast to the work done with locally applied heat, Harrison (31) has studied the effects of total body exposure to high temperature and has shown that prolonged exposure to high temperatures of male mice and rats in a hot room does not affect their fertility. He thus concludes that local application of heat to the testis is injurious because of some locally induced

vascular phenomenon.

There has been much controversy as to the actual type of damage to the germinal epithelium resulting from elevated testicular temperatures. Some workers believe the primary spermatocytes to be particularly susceptible (10) (67) while others consider spermatid development to be the most sensitive phase (14). Still others have observed both spermatocyte and spermatid dearrangement and disappearance following elevated tempeature exposure (1). This lack of agreement may possibly be attributed to the fact that these different investigators employed for their studies not only various species of animals and widely differing temperatures, but also various exposure times to the elevated temperatures.

An entirely different approach with regard to the effect of temperature on the testis was made by Herrington and Nelbach (35). They noticed a decrease in testicular and adrenal weights of the rat at 35° C. as compared to 28° C. However, associated with this testicular retardation seen at the higher temperature was a low tensile resistance of the rat's thoracic aorta, resembling that of older animals. They stated that the fact that high temperature produced testicular atrophy, associated directly with similar aortic effects, points to a direct relationship between gonadal principles and cardiovascular function.

Walton (76) observed in rabbit spermatozoa that a temperature just above that of the abdominal cavity of the rabbit resulted in instantaneous death of the spermatozoa, thus indicating a critical temperature just above normal body temperature.

4. <u>Effect of Low Temperature on Testicular Morphology</u>

Macdonald and Harrison (44) observed that a temperature of -6 and -8 °C., when applied directly to the testis for one hour, caused spermatogenic destruction with no evidence of regeneration. However, a temperature of -10 °C. applied to the scrotum for one hour was necessary to produce severe spermatogenic damage from which some regeneration could occur. He attributed these differences to the protective influence of the scrotum. Walton (76) noticed in rabbit spermatozoa that lowering the temperature below the optimum needed for maximal survival resulted in an increase in the velocity of destruction of the spermatozoa.

5. Possible Causes of Temperature-Induced Testicular Damage

There is general agreement that testicular damage, following either experimental cryptorchidism, application of heat to the scrotum, or insulation of the scrotum against loss of endogenous heat, is related to an increased testicular environmental temperature.

It appears from the work of various investigators that the progressive adaptation of the testes to a scrotal environment may have resulted in an increase in the susceptibility of the germinal cells to elevated temperatures. Fukui (25) believes that the cause of descent of the testes into the scrotum might be due to the fact that the spermatogenic protein is thermolabile at normal body temperatures. Barron (3) believes that "heat degeneration" is due to a hyperemia resulting from vasodilation. Moore (49) states that oxygen lack and accumulation of carbon dioxide due to vascular stagnation may be the cause of heat-induced testicular damage. Cross and Silver (12), however, report that oxygen tension in the testis of rabbits increases when the scrotum is warmed. They attribute this to a change in the blood vessels

representing a vasodilatation in response to the scrotal warming. Meschaks (13) showed that seminal degeneration in bulls following insulation of the scrotum is closely related to the excretion of neutral steroids in the urine; he suggests that abnormal function of the testes under these conditions might be due to excessive production of testicular and adrenal cortical hormones. Turpeinen et al. (73) studied the fate of certain enzymes of the rat testis in which spermatogenesis was suppressed by exposure to external heat at 44° C. for 20 minutes. Although there was clear evidence of histological damage to the seminiferous epithelium 24 hours after this treatment, there was no evidence of changes in the content or the distribution of hyaluronidase, succinic dehydrogenase, acid phosphatase, or alkaline phosphatase. However, 21 to 30 days later he noticed a marked decrease in the hyaluronidase concentration as well as a marked change in the distribution of both succinic dehydrogenase and acid phosphatase; the chief locus of the activity of these two enzymes had been transferred from the tubules to the interstitium.

Macdonald and Harrison (44) believe that the untoward results of low temperatures on spermatogenesis are probably caused by ischemia, which in turn may be caused by vasoconstriction produced by the low temperature.

C. THE SCROTUM

The scrotum of mammals is a structure the function of which is to regulate the environmental temperature of the testes; it is a local thermoregulator of the male mammal and controls the activity of the testis by providing an optimum environment for spermatogenesis (53). The fact that the scrotum is essential for the continuance of germ cell differentiation is shown by removal of the testes from the scrotum or by experimentally interfering with its

function (52).

In its most simple type, the scrotum is merely a thinned area of the anterior body wall; more advanced development produces a protruding pouch. Its higher form in higher mammals is a pendant sac completely isolated from the peritoneal cavity. In this well-developed form, the scrotal sac is a structure in which the testes are able to reside in a temperature environment that permits the complete expression of their functional capacities. This environment has been shown to be maintained several degrees below the general body temperature.

An investigation of the temperature of the abdominal cavity, in comparison with that of the scrotum, has been made on rats, guinea pigs, and rabbits with the result that a significant difference was found to exist (54). In each case, it was found that the scrotal temperatures were lower than the corresponding peritoneal temperatures. This would be expected if it is a higher temperature of the peritoneal cavity that causes testicular degeneration or, conversely, if it is a necessarily lower temperature that is essential to permit the testis to remain normal and active.

D. THE VASCULARITY OF THE TESTIS

It is important to establish the range of temperature in which testes situated in a scrotum normally function, for the peculiar vascular pattern of the mammalian testis indicates in itself that efficient thermoregulation of the testis is vital.

1. Comparative Anatomy

Harrison and Weiner (32) have studied the testes of various mammals and have observed that there are fundamental differences in their vascular supply

and that the vessels display remarkable complexity both in their venous and arterial courses. Some of their observations are as follows:

a. Rat

There is marked coiling of the testicular artery in its course to the testis. The artery before reaching the testis is about 0.2 mm. in diameter and forms about 30 closely packed loops on the anterior surface of the testis before giving off any branches. The veins of pampiniform plexus are few, of small diameter, and do not have a very close relationship to the testicular artery.

b. Mouse

The testicular artery in passing to the testis is only slightly undulating and shows the least convolutions of any mammal studied. It also has the smallest diameter (0.1 mm.) and is completely surrounded by testicular veins without the intervention of connective tissue.

c. Rabbit

In passing to the testis the artery which is 0.3 mm. in diameter shows about 30 loops which are packed almost as tightly as in the rat. The testicular artery is almost completely surrounded by testicular veins, but is separated from them by connective tissue.

d. Guinea Pig

The artery convolutes only slightly in its course to the testis to form about 10 complete loops. The artery is 0.2 mm. in diameter in adult animals, and is intimately related to the veins of the pampiniform plexus which surround it on all sides in direct contact with it.

e. Dog

The testicular artery in the spermatic cord is 0.8 mm. in diameter and forms 25 to 30 loosely packed irregular convolutions before reaching the testis. The veins of the testis converge toward the posterior border to form the pampiniform plexus. These veins then pass up the cord, surrounding the convolutions of the testicular artery as an anastomosing network.

2. Functional Anatomy

As has been shown there are wide variations in the different species both in the calibre and the tortuosity of the testicular artery. In addition, the relationship of the testicular artery to the pampiniform venous plexus also shows much divergence. Yet there does seem to be some similarity in the vascular patterns of some mammals. Harrison (33) has shown that the testes of the dog and rat are essentially similar in their vascularization in relation to anastomoses; the cremasteric artery does not appear to enter into arterial or arteriolar communication with the testicular and deferential arteries.

The functional significance of these various vascular arrangements is thought to be related to the thermo-regulatory characteristics of the testis (32). It is well recognized that testicular temperatures are lower than those of the abdominal cavity, and that these temperature gradients vary between animals. Some of the features which could bring about these temperature gradients and also the observed species differences are as follows: 1) An increase in the numberof convolutions and a decrease in the calibre are features of the testicular artery which would conceivably slow down the

blood flow and give the arterial blood more time and surface for cooling. (2) The smaller the testis of the animal, the greater would be its cooling capacity in relation to its weight. (3) The rather long passage of the testicular artery on the surface of the testis would contribute towards increasing the degree of cooling of the arterial blood before it reaches the interior of the organ. (4) The relationship of the testicular artery to the pampiniform venous plexus would also play a major role. Since the venous blood leaving the testis would tend to be cooler than the blood in the testicular artery leaving the abdomen, the close proximity of the two streams in the spermatic cord would serve to bring about a precooling of the descending arterial blood flow, thereby serving to enhance the cooling features of tortuosity and small calibre. Such an arrangement would have the effect of bringing the testis to a temperature below that of the abdomen. With precooling of the testicular artery, there would also be preheating of the testicular veins.

This vascular mechanism may also have a role in protecting the testis from increases in body temperature. Thus, in hyperpyrexia such as might occur with some generalized diseases or feverish conditions of the body, it could be expected that the tortuosity of the artery and its precooling by returning veins, together with other features improving heat dissipation, would serve to minimize testicular heating from the body.

3. Counter-Current Exchange Phenomenon

According to Dahl and Herrick (13) the anatomic arrangement of the blood vessels of the testis in many mammals suggests that the principle of counter-current exchange is valid in these animals, and that, in conjunction

with the position of the testes and the structure of their coverings, it aids in maintaining a testicular temperature suitable for spermatogenesis. By the phrase "counter-current exchange" these investigators meant the principle of transfer of heat between fluids of different temperatures flowing in opposite directions in adjacent conduits; ; heat is lost from the warm fluid into the cooler fluid. The efficiency of such a system at given temperatures depends on the intimacy of the conduits, and on the length of time that the contained fluids are in association, hence on the rate of flow and on the length of the associated conduits. Increasing the surface across which heat can be conducted, as by increasing the number of tubes in proximity to each other, will increase the exchange of heat. These investigators thought that because of the arrangement of the numerous veins of the pampiniform plexus about the internal spermatic artery, an anatomic counter-current exchange unit was present which could result in a reduction in the temperature of the blood within the artery.

Waites and Moule (75) observed that blood flowing through the internal spermatic artery of the ram cooled by approximately 5 °C. between the aorta and the dorsal pole of the testis where testicular temperatures were about 34 °C. Most of the cooling occurred in the coiled portion of the artery in the spermatic cord where the venous blood returning from the testis through the pampiniform plexus was seen to warm by a similar amount; this area was that part of the spermatic cord where the internal spermatic artery was in closest contact with the pampiniform veins. Yet, even though they concluded this to be an efficient heat-exchange system, they could not state that this vascular heat-exchange <u>per se</u> could actively

regulate the temperature of the testis. Indeed, it only serves to cool the testis when the returning venous blood is cooler than the arterial inflow, and this relationship can be maintained only if heat is being lost in the scrotum. This latter point was also emphasized by Crew (11).

E. THE METABOLISM OF EJACULATED SPERMATOZOA

1. Carbohydrate

Mammalian semen contains fructose, its concentration varying from a few mgm. per cent in boar to 100 mgm. per cent in bulls; this sugar serves the spermatozoa as their natural nutrient (47). At the site of its origin in the testis and in the epididymis, the semen contains very little fructose but acquires it during its passage through the whole male generative tract, from the accessory glands of reproduction, mainly the seminal vesicles. Thus, the reducing carbohydrate in the seminal plasma of the ram and bull is fructose, which is readily converted anaerobically by the spermatozoa to lactic acid, thus providing an important source of energy for the sperm cells (45). The process of fructose formation is initiated and controlled by the testicular hormone testosterone; a hormonal deficiency due to castration causes a decrease or disappearance of seminal fructose, but treatment with testosterone promptly restores the ability of the accessory glands to produce fructose (47). Bartlett (4) stated that dog semen was characterized by very low concentrations of fructose. However, studies on sperm metabolism showed that dog spermatozoa can utilize added fructose under anaerobic conditions very rapidly.

In bull and ram semen the anaerobic survival of spermatozoa is closely dependent upon the presence of fructose. If ram or bull spermatozoa are

washed free from the fructose-containing seminal plasma and thus deprived of fructose, they soon become immotile under anaerobic conditions (45). However, their survival can be extended considerably by the addition of a glycolyzable sugar.

Under aerobic conditions, on the other hand, spermatozoa can survive temporarily even after the removal of fructose-rich seminal plasma. The ability of these spermatozoa to take up oxygen is of rather short duration but can be maintained for a considerable length of time by the addition of fructose or lactate. These findings (45) suggest that the metabolism of fructose or the process of fructolysis plays an essential role in the survival of mammalian spermatozoa under both anaerobic and aerobic conditions.

Washed spermatozoa can also metabolise glucose and mannose, as well as fructose, to lactic acid (72). The metabolic degradation of these three sugars is apparently accomplished by the same hexokinase-catalyzed reaction with adenosine triphosphate. The enzyme hexokinase contained within the sperm cell, catalyzes with almost the same efficiency the transfer of phosphate groups from ATP to fructose, glucose, or mannose. It has also been shown that ram and bull spermatozoa can metabolize glycerol and sorbitol under aerobic conditions, breaking them down to lactic acid (46).

Balogh and Cohen (2) state that spermatozoa thrive under anaerobic conditions by utilizing extracellular substrates found in semen. Under these anaerobic conditions glycolysis is the metabolic pathway acting as the major energy source. He supports this by having demonstrated cytochemically the presence of glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase activity.

The oxidative phase of sperm metabolism whereby additional energy is obtained by the oxidation of lactic acid to carbon dioxide and water apparently takes place through the Krebs tricarboxylic acid cycle. Balogh and Cohen (2) have demonstrated the presence of the following oxidative enzyme systems in mammalian spermatozoa: DPNH diaphorase, TPNH diaphorase, succinic dehydrogenase, malic dehydrogenase, and isocitric dehydrogenase.

Lardy and Ghosh (40) have discussed the presence of a labile, lipid soluble substance in ejaculated spermatozoa which when added to bovine epididymal spermatozoa will stimulate both the rate of respiration and anaerobic glycolysis. This substance which apparently reverses the normal Pasteur effect of spermatozoa is known as the "metabolic regulator."

2. Protein

Polypeptides and proteins of low molecular weight are present in mammalian semen. Free amino acids are also present and apparently arise through the breakdown of proteins after the semen is ejaculated. Lundquist (42) observed that the concentration of free amino acids in freshly ejaculated semen is very low, and that a rapid increase in the concentration takes place on incubation at 37° C. or even at room temperature. Among the amino acids found in the semen are alanine, glycine, valine, leucine, isoleucine, serine, threonine, aspartic and glutamic acids, and small amounts of tyrosine, phenylalanine, proline, and lysine. It was found that this increase in the amino nitrogen level of semen after incubation at 37° C. was due to a fibrin-like proteolytic enzyme which was formed in the prostate gland (43). Inasmuch as this same amino acid picture can be obtained by incubating this "seminal proteinase" enzyme with the fibrin-like material

from freshly ejaculated semen, it is believed that the majority of amino acids found in semen is formed through the breakdown of seminal fibrin.

Bhargava (6) observed a rather high rate of incorporation of radioactive amino acids into the proteins of bull spermatozoa. Since these cells are said to be virtually devoid of ribonucleic acid, the suggestion arose that RNA was not essential for protein synthesis, at least in these cells. However, Martin and Brachet (48) contradicted these results by employing radioautography to assay the labeled amino acid uptake. Their finding that the incorporation of labeled amino acids occurs in the cell debris rather than in the spermatozoa ruled out the possibility of bull spermatozoa being able to possess high rates of protein synthesis while possessing a low RNA content.

Tosic and Walton (71) observed that bull spermatozoa in the presence of oxygen can oxidatively deaminate certain L-amino acids (L-tryptophane, L-phenylalanine, and L-tyrosine) with the formation of ammonia and hydrogen peroxide. Under anaerobic conditions, these substances were not produced. It is known that hydrogen peroxide is very toxic to spermatozoa *in vitro* because of their lack of catalase (7), thus leading to subsequent inhibition of sperm respiration and motility.

Roy and Bishop (64) observed that relatively large amounts of glycine had a beneficial effect on the survival of bull, boar, and stallion spermatozoa, but they were unable to show that it was metabolized. Subsequently, Flipse (24) showed that C^{14}- glycine was metabolized by washed bull spermatozoa to formate and carbon dioxide. He also observed that glycine is capable of reducing CO_2 production from labeled glucose and therefore postulated that

glycine utilization may have a sparing effect on glycolysis in bovine spermatozoa.

F. THE METABOLISM OF THE NORMAL TESTIS

Wolf and Leathem (80) state that the testis of the rat consists of approximately 85% water and 15% solids. The solids of the testis of adult rats are 65-70% protein and about 30% lipid. Fresh testicular tissue has also yielded detectable glycogen.

1. Carbohydrate

The metabolism of the testis of the rat is similar to that of the brain in that both tissues show high anaerobic glycolysis (20). However, it has been shown that the testis does differ from the brain, liver, and kidney in three main ways. First of all, the testis shows a fairly high aerobic glycolysis which is not inhibited by added lactate. Secondly, the testis carries on a considerable metabolism of pyruvate under anaerobic conditions. Finally, the testis differs from other tissues in forming, in anaerobic experiments without glucose, a considerable amount of acid which is not lactic acid.

Elliott et al. (20) has also shown that lactate is quite rapidly oxidized by the testis of the rat to pyruvate which is in turn rapidly metabolized by the testis. In the absence of glucose, addition of pyruvate increases the oxygen uptake considerably; in the presence of glucose, the actual oxygen uptake remains about the same, but the oxidation processes are probably increased since pyruvate acts as a hydrogen acceptor in addition to oxygen. In both cases there is increased lactate formation due to the reduction of pyruvate.

In anaerobic experiments with the testis and pyruvate, there is a considerable evolution of carbon dioxide. In this respect the testis differs from other tissues, such as the liver, brain, and kidney.

As in other normal tissues, succinate is rapidly oxidized by the testis, its addition causes a large increase in oxygen uptake, especially in the absence of glucose. Fumarate and malate are oxidized in the testis only to a small extent since only small increases in oxygen uptake have been observed.

In the presence of glucose, addition of acetate has been seen to cause a small increase in the oxygen uptake, while in the absence of glucose, there was no increase in the uptake of oxygen. It seems that acetate oxidation occurs slowly in the testis and is dependent on simultaneous carbohydrate oxidation. Acetate is apparently not an intermediate step in the oxidation of pyruvate since the oxidation of pyruvate, but not of acetate, continues in the absence of glucose.

Dickens and Greville (17) have observed that the testis of the rat shows a large decrease in respiration when deprived of glucose. Thus, the testis seems to show a marked dependence on the ~~suppoly~~ supply of this sugar. In contrast, the kidney, liver, and spleen suffer relatively little fall in respiration when deprived of glucose. As a result, it is thought that these three organs have a dietary supply of glucose which is not available to the testis. These investigators have also stated that the testis shows a slight tendency to convert fructose into lactic acid. The marked decrease in respiration of the testis in the absence of glucose can be prevented by the addition of fructose.

Tepperman et al. (68) has studied the metabolism of the rat testis in vitro and has hypothesized that the germinal component of the normal testis is characterized by a low oxygen uptake in the absence of added substrate such as glucose. He also observed that the transplanted crytorchid testis shows a highly significant increase in oxygen consumption in the absence of added substrate as compared with the normal scrotal gland.

2. Protein

Although Fukui (25) in 1923 first suggested the importance of testicular proteins, it has only been in recent years that the actual functional relationship of these proteins has undergone thorough investigation. Neame (57) believes that the ability of a tissue to take up amino acids should be considered as a measure of the metabolism and growth of the tissue and should be thought to be greater in those tissues whose cells are often in the process of division or in which there is a specialized need to transport or conserve amino acids. Such a tissue is the testis, which is composed mainly of cells designed for replication.

Thorsteinsson (69) has observed a strong peptidase activity in extracts of the testis, epididymis, epididymal sperm, and the accessory glands of the rabbit. Some other enzymes found both in mammalian spermatozoa and testicular tissue include those of the glycolytic system, the cytochrome system, and the tricarboxylic acid cycle (8).

Much in vitro work has also been done with regard to testicular protein biosynthesis. Davis et al. (15) has sown that in the testis of the adult rat, maximal incorporation of radioactive lysine into protein occurred at 32°C., thereby indicating that the protein-synthesizing systems of the rat

testis are very heat-labile. On the other hand, Hall (28) has demonstrated that both steroid biosynthesis as measured by the incorporation of acetate-1-C^{14} into testosterone-C^{14} *in vitro* and protein biosynthesis as measured by the incorporation of L-lysine-U-C^{14} into protein *in vitro* were found to be maximal in slices of rabbit testis at 38° C. Both steroid and protein biosynthesis were significantly lower at 40° C. than at 38° C. It has also been shown that the interstitial cell stimulating hormone increased the incorporation of valine-1-C^{14} and tryptophan-1-C^{14} into protein by slices of the testis of the rabbit when the hormone was administered *in vivo* or added *in vitro*; the incubation temperaute was 37.5 °C. (29). The response of slices of testis to ICSH *in vitro* was inhibited by chloramphenicol and puromycin, compounds which are known to be specific inhibitors of protein synthesis; it is believed that both substances affect the transfer of amino acids from soluble RNA to protein.

3. Androgen Production

The testis performs two primary functions: the proliferation of spermatozoa and the secretion of steroid hormones. The latter determines the physiological state of the accessory ducts and glands. It is generally believed that testosterone, the chief hormone of the testis, is synthesized and secreted by the interstitial cells of Leydig.

The hypophysis of the male liberates follicular-stimulating hormone (FSH), luteinizing hormone (LH), or interstitial cell stimulating hormone (ICSH), and prolactin. When extracts containing FSH are administered to a hypophysectomized rat, spermatogenesis is maintained at least through the secondary spermatocyte stage (72). However, the sex accessory organs of of these animals remain atrophic, indicating that the cells of Leydig have

not been stimulated to produce androgen. Complete maintenance of the spermatogenic function in the hypophysectomized rat is said to require both FSH and ICSH. Extracts containing ICSH stimulate the cells of Leydig, as is reflected in the growth and functional activity of the sex accessories. In fact, it is generally believed that three hormones, FSH, ICSH, and androgen participate in the normal development and function of the seminiferous tubules. Hall and Eiknes (29) have shown that the incorporation of acetate-1-C^{14} into testosterone-C^{14} in vitro is increased by interstitial cell-stimulating hormone and follicle-stimulating hormone added in vitro or by interstitial cell-stimulating hormone and human chorionic gonadotropin administered in vivo.

Gospodarowicz and Legualt-Demare (27) measured the incorporation of acetate-1-C^{14} into cholesterol, progesterone, and androstenedione by rat testis in vitro at 27° and 38° C. They found a greater specific activity of cholesterol-C^{14} and progesterone-C^{14} at 38° as compared to that at 27° C., but a decrease in specific activity of androstenedione-C^{14} at the higher temperature. These findings suggest a decrease in the production of testicular androgen (androstenedione) at 38° as opposed to 27° C. and appear to be contrary to those results reported by Hall (28).

The most significant metabolic action of androgens is the stimulation of protein anabolism. Testosterone has been shown to increase the nitrogen retention of castrated male adult rats without increasing the nonprotein nitrogen of the blood, and produce at least a temporary increase in body weight (60). This suggests that the hormone causes a true storage of nitrogen in the form of tissue protein. In the dog, androgens have been reported to increase the synthesis of proteins and decrease the rate of catabolism of

amino acids (72).

Niemi and Ikonen (59) have identified the activities of many oxidative enzyme systems in the Leydig cells of the rat testis. These enzymes are as follows: diphosphopyridine and triphosphopyridine nucleotide diaphorases, and succinic, lactic, glutamic, alpha-glycerophosphate, beta-hydroxybutyrate, and steroid-3-beta-ol-dehydrogenases. These oxidative enzymes represent many different metabolic pathways: succinic dehydrogenase could be considered indicative of the activity of the Krebs cycle; glutamic dehydrogenase is concerned with the oxidative deamination of the amino acids; two of the enzymes (alpha-glycerophosphate and lactic dehydrogenase) are involved in glycolysis; beta-hydroxybutyrate dehydrogenase takes place in the oxidation of fatty acids; and, steroid-3beta-ol-dehydrogenase catalyzes an early reaction in the formation of the nonbenzoid steroid hormones. DPN and TPN diaphorases together give the total activity of the pyridine nucleotide linked dehydrogenases. These investigators then observed that both hypophysectomy and the inhibition of the production of ICSH by testosterone injections caused a very pronounced decrease in the activity of beta-hydroxybutyrate dehydrogenase, and thus concluded that the fatty acid oxidation in the Leydig cells is intimately correlated with their hormonal activities.

4. Effect of Temperature on Testicular Metabolism

Degeneration of the germinal epithelium of the testes as a result of exposure to temperatures higher than those normally found in the scrotum has long been known (50). Assuming that the cooling mechanism of the scrotum plays an essential role in the regulation of spermatogenesis and testicular metabolism, it might be expected that abdominal increases in

temperature would result in changes in the metabolic activity of the tissue. Ewing and Vandermark (22) observed the in vitro metabolic activity of rabbit testis exposed to abdominal temperatures for 2, 6, and 24 hours in vivo. They noticed that the metabolic activity increased with the shorter exposure and then ~~and then~~ significantly decreased as compared to normal testicular tissue. Tissues exposed for 24 hours contained 12% less glucose and 27% less lactic acid than controls, thus suggesting that spermatogenic arrest which results from exposure of the testis to elevated temperatures may be caused by reduced levels of substrate in the tissue. Experiments were then carried out in which the testicular tissue was subjected to increasing abdominal-scrotal temperature gradients. Here, the results were a lowered metabolic activity as measured by a decreased lactic acid accumulation and oxygen uptake; however, there was an increased glucose uptake initially. The possible explanation for this is that large amounts of glucose were removed from the tissue by the increased metabolic rate during exposure to the elevated temperature of the abdomen and that the depleted supply was replaced from the exogenous source. These same investigators then carried out in vitro metabolic testicular studies with in vitro elevations in temperature (23). They found that when an adequate amount of substrate (0.055 M. glucose) was present, the enzyme systems of the testis of the rabbit were not deleteriously affected by an increased temperature (36.5°C. vs. 39.0°C.) in vitro. An increased temperature to 39.5°C. in vitro caused an increase in glucose uptake of the isolated, perfused rabbit testis during the first two hours of perfusion followed by a substantial decrease in the amount of glucose provided to the tissue during

the second and third hours of perfusion. These results again suggest that the effect of temperature higher than scrotal temperature on spermatogenesis is preceded by a transient increase and then a decrease in the metabolic activity of the tissue.

Davis et al. (16) has studied the effect of both temperature and glucose on testicular protein labeling of the rat. He observed that the addition of 0.009 M glucose increased the incorporation of radioactive lysine into protein of slices of rat testis and head of the epididymis by 600 and 160%, respectively. He also observed that in the presence of glucose, the incorporation of labeled lysine into testicular protein was approximately equal at both 34 and 37.5°C., whereas without glucose, protein labeling at 37.5°C. was one-half that observed at 34°C. These data suggest that the inhibitory effect of increasing temperature on the protein-synthesizing systems of the rat testis (15) may be partially reversed by the addition of glucose.

Hill (36) noticed that the grafting of ovaries into the ears of castrate male mice caused the normal growth and secretion of the seminal vesicles and prostate. He then observed that the temperatures of the ears of mice approximates the temperatures of the scrotum, averaging 5-6°C. below that of the abdomen. This suggested that temperature may be the controlling factor in the output of male hormone by an ovary grafted in the ear of castrate mice. On the other hand, Hoffman et al. (37) noticed that a low temperature of 18.5°C. resulted in significant adverse effects on testicular weight accompanied by rapid losses in ascorbic acid content and marked increases in cholesterol concentration. Histochemical techniques revealed

increases in tubular lipids and changed distribution.

G. EFFECT OF TEMPERATURE ON THE KINETICS OF METABOLIC REACTIONS

Prosser (63) has stated that temperature, perhaps more than any other environmental factor, has multiple and diverse effects on living organisms. It limits the rate of chemical reactions and hence, is a major factor in growth and metabolism.

1. Optimum Temperature

Temperature is one of the most influential and important factors governing reaction mechanisms specifically in biological processes. The optimum temperature is generally considered to be that at which the maximum rate of the reaction occurs (30). It has long been believed that enzymatic processes show acceleration when the temperature is raised, but will slow down rapidly when a maximum point is reached because of thermal destruction of the enzyme protein molecule itself, in which case, irreversible changes occur in the molecular configuration of the enzyme, greatly affecting its activity and ultimately causing its arrest. This optimum temperature has been shown to actually be a range of temperatures, rather than a sharp point, since living systems are accustomed to certain degrees of change in their environmental temperature, which in turn, influences their internal temperature; thus, their metabolic processes have acquired, during the course of evolution, a certain flexibility in their response to changes in temperature over a limited range. It is important to maintain the optimum temperature in enzymatic processes, because should it fall or rise, the inherent energy of the reactive system would be either

reduced or increased, thereby causing considerable variation in an important factor of the reaction mechanism.

2. Effect of Temperature on Enzymes

It has been shown for some enzymes that an equilibrium exists between the native, active enzyme and its inactive, denatured form (38); this equilibrium may be influenced by temperature. At any temperature the actual effect observed is the result of both the activation of the enzyme as well as the equilibrium governing the amount of active enzyme. Thus, at low temperatures with respect to the optimum, activation of the enzyme predominates, and denaturation is negligible. At relatively high temperatures, reversible denaturation of the enzyme predominates.

3. Heat Inactivation of Enzymes

The influence of temperature on an enzymatic reaction is generally associated with two different effects operating simultaneously (18); there is an increase in the initial velocity or true catalytic activity of the enzyme, together with a destruction of the enzyme at higher temperatures, producing a continuous fall in the concentration of active enzyme. The optimum temperature is determined by the balance between the effect of temperature on the rate of the enzyme reaction and its effect on the rate of destruction of the enzyme.

The rate of inactivation of enzymes in solution increases rapidly with the temperature. This has been shown by determining the amount of active enzyme remaining after a given exposure to a certain temperature and then cooling it to a standard temperature with the subsequent addition of substrate. It has been shown that temperature inactivation may become appreciable at a temperature as low as 30° C. and for the majority of

of enzymes is very marked at 50-60° C. The misconception of arbitrarily choosing 37.5° C. as a working temperature in enzyme studies is obvious in view of the partial inactivation of some enzymes at this temperature (66).

The inactivation of enzymes by heat is nearly always due to the denaturation of the enzyme protein. The term denaturation is used to indicate disorganization of the natural rigid protein molecule. This can occur through either unfolding of the peptide chain or through dissociation into smaller units which may or may not be unfolded (39). The normal protein pattern can be broken up by rupturing the secondary intramolecular bonds which maintain the native protein in its folded state. Among these secondary bonds are the following: (1) Hydrogen bonds between the carboxyl oxygens and the amide hydrogens of the peptide bonds. (2) Hydrophobic bonds referring to the interactions between the nonpolar groups of proteins in water. Many of the residues of proteins carry a nonpolar side chain such as alanine, valine, leucine, isoleucine, proline, and phenylalanine. Inasmuch as the interaction of nonpolar groups with water is unfavorable, there is a tendency for these nonpolar groups to contact each other, with an accompanying decrease in their interactions with water (39) (65). (3) Salt bridges or ionic bonds which could form between positively charged (lysine and arginine) and negatively charged (glutamic and aspartic acids) amino acid residues. (4) van der Waals forces or dispersion forces which increase the stability of the protein (5) Intramolecular chemical bonds such as the disulfide bond of cystine.

Much work on enzyme kinetics as a function of temperature indicates that many enzyme reactions follow the Arrhenius equation over a wide temperature range up to temperatures where heat inactivation of the enzyme becomes apparent. The similarity between the high values for the activation energies of enzyme destruction and protein denaturation has been pointed out by many workers, and has been interpreted as evidence for the protein nature of enzymes and for the identity of the mechanisms of heat denaturation of protein and heat inactivation of enzymes (66). Thus, it is indicated that both the denaturation of proteins and the inactivation of enzymes result from the breaking of a large number of bonds, such as hydrogen bonds. This is consistent with an accepted picture of denaturation as an opening up of the molecule by unfolding or separation of adjacent portions of peptide chains.

4. Arrhenius Theory of Reaction Rates

The general formulation of the effect of temperature on reaction rates was given by Arrhenius. He postulated that not all the molecules in a system are capable of reacting and that only those which have a high energy content, or only those molecules which have sufficient energy of activation, are capable of reacting. In other words, the Arrhenius theory of reaction rates states that only those molecules possessing more than a certain amount of kinetic energy are said to be activated (78).

It is thus assumed that there is an equilibrium between inactive and active molecules with the constant K being designated as the equilibrium constant. The effect of temperature is on the equilibrium constant for a chemical reaction and is given by the van't Hoff equation,

$$2.3 \log K = C - \frac{\Delta H}{RT}$$

where ΔH is the change in heat of the reaction in calories per mole, R is the gas constant equal to 1.98 cal per mole per degree, and T is the absolute temperature. C is an integration constant.

Arrhenius assumed that the energy of activation (E_a) could be obtained for rate processes in the same manner as H for equilibrium processes. Inasmuch as it is known that an increase in temperature will increase the rate of a chemical reaction, Arrhenius thought that the effect of temperature on the equilibrium was one of determining the rate of formation of active molecules.

The Arrhenius equation relating a velocity constant K to absolute temperature is as follows:

$$2.3 \log k = B - \frac{E_a}{RT}$$

where E_a is the energy of activation, and B is a constant which is a qualitative expression of the frequency of collisions of the participating molecules, thus contributing to the lowering of their energy of activation. For two temperatures, the following equation was presented:

$$2.3 \log \frac{k'}{k''} = \frac{E_a}{R} \left(\frac{1}{T''} - \frac{1}{T'} \right)$$

As a rule, this equation describes the data for ordinary chemical reactions. In general, increased temperatures favor the formation of active molecules,ie, those with suf-icient energy of activation to react, and this process is satisfactorily described by the Arrhenius equation.

CHAPTER II

MATERIALS AND METHODS

A. ANIMALS

The animals employed in these experiments were as follows: (1) Adult male Sprague-Dawley rats 55-65 days of age and weighing 210 to 255 grams. (2) Adult male Swiss mice 7 weeks of age and weighing 18 to 24 grams. (3) Adult male Golden Syrian hamsters 7-8 weeks of age and weighing 80 to 97 grams. (4) Adult male New Zealand White rabbits 6 months of age and weighing 3.75 to 4.00 kilograms. (5) Adult male Hartley guinea pigs 10 weeks of age and weighing 455 to 515 grams. (6) Adult male Mongrel dogs 2-5 years of age and weighing 7.5 to 10.5 kilograms.

The rats, mice, hamsters, rabbits, and guinea pigs were obtained from the Abrams Small Stock Breeders, Chicago, Illinois and were housed at a constant temperature of 72°F. Their diet consisted of Rockland laboratory chow fed ad libitum. The dogs were obtained from the animal quarters of the Stritch School of Medicine of Loyola University, Chicago, Illinois and were fed Rockland dog food with additional meat and enriched bread ad libitum.

B. TRACER

The tracer employed was L-lysine monohydrochloride uniformly labeled with C^{14}. The L-lysine-U-C^{14} used had a specific activity of 5.8 mc/mmole and was purchased from the Nuclear-Chicago Corporation.

C. PREPARATION OF THE TISSUES

All animals with the exception of the dogs were sacrificed by decapitation and taken into the cold room where the temperature was 4°C. The

dogs were anesthetized with 50 mg/kg of pentothal sodium. The testes were removed and weighed to the nearest milligram on a Roller-Smith torsion balance. The testicular capsule was removed and slices made with the aid of a Stadie-Riggs microtome. While the excisement and slicing of the testicular tissues were being carried out, previously prepared Warburg flasks containing 3.0 ml of Krebs-Ringer bicarbonate buffer at pH 7.4 in the main chamber and 0.2 ml of L-lysine-U-C^{14} containing 2.5×10^5 counts/minute in the side-arm were equilibrating to the temperature of the cold room. The Krebs-Ringer bicarbonate buffer had been pregassed with 95% O_2 and 5% CO_2 for ten minutes. To each Warburg flask 150 to 200 milligrams of testicular slices were added and swirled. The flasks were then rapidly taken from the cold room and placed on the manometers.

D. INCUBATION OF THE TISSUES

The flasks were gassed for ten minutes with 95% O_2 and 5% CO_2 while adjusting to the temperature of the bath. Following this equilibration period, the labeled lysine was added and the incubation carried out for the desired time at 140 oscillations per minute. The final concentration of L-lysine-U-C^{14} in the incubation flask was 1.8×10^{-5}M. Incubation of tissue slices was carried out simultaneously at temperatures ranging from 26°C. to 44°C. This was accomplished by using several temperature-controlled water baths surrounding a main Warburg apparatus. The incubation period was terminated by adding the contents of each Warburg flask into a test tube containing 0.3 ml of 5N perchloric acid. The test tubes were then allowed to stand under an exhaust hood for 15 minutes. During this period the tubes were occasionally mixed with the aid of a Vortex jr. mixer. The protein

samples were then centrifuged at 600xg for 10 minutes, and the supernatants were discarded.

E. EXTRACTION OF PROTEIN

1. Solvents

Protein extraction was carried out with the successive addition of 0.6N perchloric acid, cold 5% trichloroacetic acid (TCA), hot 5% TCA at 87°C. for ten minutes, 95% ethyl alcohol, 100% ethyl alcohol, 2:1 chloroform: methanol, benzene, and finally, anhydrous ethyl ether (twice).

2. Homogenization and Centrifugation

After the addition of each solvent, the sample was homogenized with a motor driven Teflon pestle or mixed with a Vortex jr. mixer. The sample was then centrifuged at 600xg for ten minutes, and the supernatant discarded.

3. Desiccation

Following the final extraction with anhydrous ethyl ether, the tubes were initially dried in a desiccator which was evacuated with a suction apparatus attached to a tap outlet. Complete dryness was obtained by allowing the tubes to stand at room temperature overnight. Throughout this procedure the mouth of the tubes was covered with filter paper held on by rubber bands so as to prevent any contamination with foreign matter from the atmosphere.

F. PREPARATION OF PROTEIN PLATES

The resulting fine white protein powder was then added by means of a spatual to the center-well of a stainless steel planchet. Approximately 4-6 milligrams of the protein powder was added to each planchet from the corresponding tubes.

The protein was then compressed into a thin layer by placing a stainless steel pestle over the powder and applying one sharp blow with a hammer.

G. MEASUREMENT OF RADIOACTIVITY

The plates were then assayed for radioactivity by using a Nuclear-Chicago apparatus consisting of a Model C-110B Automatic Sample Changer, a Model 183 Scaling Unit, and a Model C-111B Printing Timer.

Following a correction for self-absorption, the uptake of L-lysine-U-C^{14} into tissue protein was expressed in terms of specific activity (counts per minute per milligram dry weight protein).

H. IN VIVO MEASUREMENT OF PERITONEAL AND SCROTAL TEMPERATURES

A Yellow Springs Tele-Thermometer with a temperature range of 20° to 45 °C. was used to obtain the peritoneal, scrotal, and testicular temperatures of the mature male rat, mouse, hamster, rabbit, guinea pig, and dog. (The actual temperature was derived after calibrating the Tele-Thermometer readings with a series of known temperature values.) For the peritoneal temperature determinations, a hyperdermic probe was inserted through the abdominal wall to a point within the approximate vicinity of the liver. The scrotal temperature was measured by inserting the hyperdermic probe immediately beneath the epidermis on the anterior side of the animal's scrotum. Testicular temperature measurements were obtained by gently holding the testes taut and then inserting the probe through the scrotal skin and tunica albuginea into the parenchyma of the testis. There was no significant difference between scrotal and testicular temperatures in any of the species employed in the present studies.

While obtaining these in vivo temperatures, all animals were unanesthesized with the exception of the dogs who received 50 mg/kg pentothal sodium via intramuscular injection.

CHAPTER III
RESULTS

A. EFFECT OF TEMPERATURE ON THE INCORPORATION OF L-LYSINE-U-C^{14} INTO PROTEIN OF TESTIS SLICES OF THE RAT, MOUSE, HAMSTER, RABBIT, GUINEA PIG, AND DOG

The incorporation of radioactive lysine into testicular protein of the adult male rat, mouse, hamster, rabbit, guinea pig, and dog is presented in Chart 1. In each case, the incubation temperatures were 32°C. and 37.5°C., and the time of incubation was one hour. The gas phase was 95% O_2 and 5% CO_2. Under these conditions it was found that in the testis of the rat, mouse, and hamster respectively maximal incorporation of L-lysine-U-C^{14} into protein occurred at 32°C; there was a marked decrease in protein labeling when the incubation temperature was increased from 32° to 37.5°C. The actual mean values for the specific activity (in terms of counts per minute per milligram dry protein) for 32° and 37.5°C. respectively were as follows: (1) for the rat, 478 and 288; (2) for the mouse, 3361 and 2681; and (3) for the hamster, 2749 and 2330. On the other hand, in the testis of the rabbit, guinea pig, and dog, maximal incorporation of L-lysine-U-C^{14} into protein occurred at 37.5°C. In contrast to the results seen with the rat, mouse, and hamster, there was a marked increase in protein labeling as the incubation temperature was increased from 32° to 37.5°C. Here, the actual values for the mean specific activity were as follows for 32° to 37.5°C. respectively: (1) for the rabbit, 1772 and 2279; (2) for the guinea pig, 3202 and 4535; and, (3) for the dog, 893 and 1220. Moreover, upon incubation of the testes from

all six animals at 44° C., very little radioactive amino acid was found to be incorporated into protein. The mean specific activity values for this temperature were as follows for the rat, mouse, hamster, guinea pig, rabbit, and dog respectively: 14, 114, 88, 274, 218, and 65. These values are negligible when compared to corresponding values for the incorporation of L-lysine-U-C^{14} into testicular protein at either 32° or 37.5° C.

B. KINETICS OF L-LYSINE-U-C^{14} INCORPORATION INTO PROTEIN OF RAT TESTIS SLICES AT VARIOUS TEMPERATURES

Chart 2 presents the incorporation of L-lysine-U-C^{14} into protein of testis slices of the rat at 26°, 32°, and 37.5° C. and at incubation periods ranging from 15 minutes to 120 minutes. The results indicate that as the time of incubation is increased, the incorporation of isotope into protein becomes progressively less at 37.5° C. as compared to 32° C. This indicates an increasing inhibition of the protein synthesizing system of the testis of the rat at an elevated temperature, especially one which approximates actual peritoneal temperature.

C. KINETICS OF L-LYSINE-U-C^{14} INCORPORATION INTO PROTEIN OF HAMSTER TESTIS SLICES AT VARIOUS TEMPERATURES

The time course of the incorporation of L-lysine-U-C^{14} into protein of hamster testis slices incubated at both 32° and 37.5° C. is presented in Chart 3. A progressive increase in protein labeling occurs with both temperatures from 7.5 to 60 minutes of incubation. However, a difference in lysine uptake into testicular protein does appear when taking both variables into consideration, that is, temperature and the length of incubation. It was found that at a 7.5 minute incubation, the incorporation of radioactive

lysine into testicular protein of the hamster was greater at 37.5 °C. than at 32 °C.; the respective values for the specific activity are 258±42 at 37.5 °C. and 192± 11 at 32 °C. At 15 minutes incubation, the incorporation of labeled lysine into testicular protein is almost identical at both 37.5 °C. and 32 °C (612 and 601 counts per minute per milligram protein respectively). Finally, after a 60 minute incubation, the uptake of lysine into protein was found to be greater at 32 °C. than at 37.5 °C.; here, the specific activity values are 2080±122 at 32 °C. and 1741±145 at 37.5 °C. It would therefore appear that the maximum incorporation of L-lysine-U-C^{14} into protein of hamster testis slices is a function of both incubation temperature and incubation time.

D. IN VIVO TEMPERATURE MEASUREMENTS OF THE ADULT RAT

Measurements of rat rectal, peritoneal, and scrotal temperatures were performed at 72 °F. (Chart 4). It can be seen that while there is virtually no difference in rectal and peritoneal temperatures, there does appear to be a substantial decrease in temperature from the abdominal cavity to the scrotal compartment.

E. COMPARISON OF INTRAPERITONEAL WITH SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS

Table I presents a comparison of the intraperitoneal and scrotal temperatures of the adult male rat, mouse, hamster, rabbit, guinea pig, and dog. All animals were unanesthetized with the exception of the dog who received an intramuscular injection of 50 mg/kg of pentothal sodium. All in vivo temperature measurements were taken at room temperature (72 °F) with the use of thermocouple probes. The mean intraperitoneal and scrotal temperatures of the six animals as well as the actual temperature readings

for five experiments are presented. Standard errors and probabilities are also shown. The results show that the mean abdominal-scrotal temperature differences for the rat, mouse, hamster, rabbit, guinea pig, and dog are 4.3, 4.4, 3.1, 3.1, 3.4, and 3.4°C. respectively. In all cases the intraperitoneal temperature is seen to be higher than the corresponding scrotal or testicular temperature. These temperature differences are all significant as is indicated by a probability of less than 0.001 in each case.

F. COMPARATIVE STUDY OF BOTH INTRAPERITONEAL AND SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS

Rather than compare intraperitoneal with scrotal temperature, Table II presents a comparison of the intraperitoneal temperatures of the six animals as well as a comparison of the scrotal temperatures of the same animals. It can be seen that all the abdominal temperatures are fairly close in magnitude although those of the rabbit, guinea pig, and dog are the highest. In surveying the scrotal temperatures it can be seen that the rabbit and guinea pig especially have higher temperatures as compared to the rat, mouse, and hamster. The dog is seen to have only a slightly higher scrotal temperature than the three mammals just mentioned, although it should be remembered that the dog was anesthetized; this, in turn, could possibly lower both the abdominal and scrotal temperature.

G. SIGNIFICANCE OF THE DIFFERENCES BETWEEN SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS

Table III presents a statistical evaluation of the differences in scrotal temperatures of the six animals under investigation. Included in this table are the actual scrotal temperatures of the respective mammals, the

differences in scrotal temperature, the "t" values, the degrees of freedom, and the probability values. The scrotal temperatures of each of the animals showing a decrease in protein biosynthesis with elevated incubation temperatures from 32 to 37.5 °C. (rat, mouse, and hamster) are compared with the scrotal temperatures of those animals showing an increase in amino acid incorporation into testicular protein at elevated incubation temperatures (rabbit, guinea pig, and dog). The results show that there is a significant difference in scrotal temperatures between the two groups of animals in all cases except one; the difference in the scrotal temperatures of the hamster and dog was not found to be statistically significant. However, one possible explanation for this is that the dog was anesthetized and, as a result, had a lower scrotal temperature than would normally be expected.

H. SIGNIFICANCE OF THE DIFFERENCES BETWEEN INTRAPERITONEAL TEMPERATURES IN UNANESTHETIZED ANIMALS

Table IV presents a statistical evaluation of the differences in abdominal temperatures of the six animals employed in this study. The same criteria used in Table III are expressed here. The results show that there is a significant difference between the intraperitoneal temperatures of the rat, mouse, and hamster compared with those of the rabbit, guinea pig, and dog. However, there is one exception; in comparing the abdominal temperatures of the mouse and dog, a significant difference was not found. This could again be explained by considering the possible role of the anesthetic given the dog in promoting a decrease in intraperitoneal temperature.

I. COMPARISON OF THE ONSET OF PUBERTY IN COMMON LABORATORY ANIMALS

Table V presents a comparative survey of the pertinent biological data on the six male animals used in these experiments: rat, mouse, hamster, rabbit, guinea pig, and dog. Included in this table are the strains of the respective animals, the average weight and age of the animals at the time of experimentation, their approximate onset of puberty, and their expected total life span. From this data it can be said that all animals at the time of experimentation were sexually mature.

J. HISTOLOGICAL COMPARISON OF THE TESTES OF THE ADULT RAT, MOUSE, HAMSTER, RABBIT, GUINEA PIG, AND DOG

Figure I presents the histological picture of the testes of the six animals used at the age in which they were employed in the present experiments. A. Stage VII in the cycle of the seminiferous epithelium of the rat testis, according to the description of Leblond and Clermont (41). The seminiferous tubules of the rat testis demonstrate the appearance of Sertoli cells, spermatogonia, primary pachytene spermatocytes, "acrosomal" and "maturation" phase spermatids. B. Stage VII in the cycle of the seminiferous epithelium of the mouse testis, according to the description of Oakberg (61). The seminiferous epithelium of the mouse testis is identified by the presence of the "acrosomal" and "maturation" phase spermatids. C. Section throught the seminiferous tubule of the hamster testis illustrating Stage VII in the cycle of the seminiferous epithelium, according to the description of Leblond and Clermont (41). Stage VII illustrates the normal mature appearance of the seminiferous tubules of the hamster testis

containing spermatogonia, primary spermatocytes, and spermatids. D. Section through a seminiferous tubule of the rabbit testis demonstrating the corresponding stage in the cycle of the seminiferous epithelium. E. Stage VII in the cycle of the seminiferous epithelium of the guinea pig, according to the description of Leblond and Clermont (41). The guinea pig testis is characterized by the presence of the "acrosomal" and "maturation" phase spermatids circumscribing the seminiferous tubular lumen. F. The histological appearance of the corresponding stage in the cycle of the seminiferous epithelium of the dog testis, demonstrating the "acrosomal" and "maturation" phase spermatids near the lumen of the seminiferous tubule. These data indicate that all the animals employed in the present experiments can be considered young adults with a sexually-mature appearing testis.

CHAPTER IV

DISCUSSION

It is generally recognized by numerous investigators that spermatogenesis is sensitive to relatively small increases in environmental temperature. The proposed role of the scrotum as a local thermoregulator for the preservation of testicular function has suggested that the testicular tissue, at least that concerned with the spermatogenic process is very thermolabile and is, in fact, altered by any temperature above that of the scrotum. Inasmuch as the solid tissue of the testis is composed of 65-70% protein (80), it appears that testicular protein represents the most heat sensitive constituents of the cell. Since the temperature phenomenon is believed to be the principal entity involved, it was decided to study its effect on protein biosynthesis. L-lysine-U-C^{14} was chosen as a radioactive precursor because this amino acid is known to be almost entirely utilized in the synthesis of proteins, its involvement in other metabolic pathways being minimal. Borsook (9) states that C^{14} is the most common isotope used for in vitro work because of its long half life and its ease of measurement.

Further interest in this subject arose through the conflicting observations made by two investigators. Davis and co-workers (15) found that incorporation of L-lysine-U-C^{14} into testicular protein by slices of rat testis was maximal at 32° C. In contrast, Hall (28) found that the maximal incorporation of labeled lysine into protein by slices of rabbit testis occurred at 38° C. This apparent difference between these two mammalian species thus merited

further examination and, as a result, led to a more extensive comparative investigation of protein synthesis in mammals.

The first concern was to expand the comparative aspect and to determine the effect of temperature on the incorporation of L-lysine-U-C^{14} into protein of testis slices of the mature rat, mouse, hamster, rabbit, guinea pig, and dog for an incubation period of one hour. Biological variation was minimized by utilizing separate temperature-controlled water baths which allowed for the simultaneous incubation of tissues excised from one animal. The results showed a species differentiation which could be divided into two groups. The first group was composed of the rat, mouse, and hamster in which a decrease in the incorporation of labeled lysine into testicular protein occurred as the incubation temperature increased from 32°C. to 37.5°C. Included in the second group were the rabbit, guinea pig, and dog. In the case of these three mammals, an increase in the incorporation of L-lysine-U-C^{14} into testicular protein occurred with the increase in incubation temperature from 32° to 37.5°C. Thus, there appears to be a sharp difference in protein formation from labeled lysine in three species as compared to the other three species studied. In the case of the rat, mouse, and hamster, protein synthesis is depressed by an increase in temperature. On the other hand, in the case of the rabbit, guinea pig, and dog, an elevated temperature appears to stimulate the incorporation of radioactive amino acid into protein of the testis. Yet, even though there was this species difference with regard to the "apparent optimal temperature" for protein biosynthesis, one similarity was observed. Upon incubation of the testes from all six mammals at 44°C., virtually no radioactive amino acid was found to be

incorporated into protein. Incorporation at temperatures below 32°C. has also been shown to be small in magnitude (15).

Because of this temperature difference in lysine incorporation into protein observed in the various species at one hour of incubation, it became of interest to investigate the effect of varying the exposure time of a testis slice to a given temperature and then to determine the incorporation of labeled lysine into testicular protein. In this case, the effect of incubating testis slices of the rat at 26°C., 32°C., and 37.5°C. for time periods ranging from 15 minutes to 2 hours and the subsequent determination of radioactive lysine incorporation expressed as the specific activity (counts per minute per milligram protein) were carried out. If 37.5°C. is supposed to theoretically represent too high a temperature for the normal functioning of the testis (at least in the case of the rat, mouse, and hamster), then it was thought that this temperature effect would be magnified over a longer incubation period. The results showed a progressive decrease in protein synthesis at the elevated temperature (37.5°C.) as compared to the lower temperature of 32°C. as the time of incubation was increased. This could indicate the effect of protein denaturation due to the fact that the specific activity of lysine incorporation into protein decreased with an increase of temperature from 32° to 37.5°C. As a result, it could probably be stated that thermolabile protein-synthesizing system was operating here (at least in the rat), since a greater quantity of testicular protein appears to be adversely affected by an increase in the time of exposure of the testicular tissue to an elevated temperature.

The next parameter investigated was a kinetic study of the incorporation

of labeled lysine into protein of the hamster testis slices at 32° C. and 37.5° C.; this was done for two reasons. First, it has already been stated that at a one hour incubation, the incorporation of L-lysine-U-C^{14} into testicular protein of the hamster is similar to that of the rat in that both species show a decrease in protein synthesis with an increase in temperature from 32° C. to 37.5° C. As a result, it was thought that by increasing the incubation time, this temperature effect on testicular protein labeling of the hamster would be magnified. Thus, this would serve as a duplicate kinetic experiment for those species showing a decreased lysine incorporation with elevated temperatures. Secondly, although it was easily seen that there was a progressive decrease in protein synthesis in the rat at increasing temperatures from 32° to 37.5° C. and at incubation periods ranging from 30 to 120 minutes, the effect at a 15 minute incubation period was not so distinct. In fact, it almost appears that the specific activity of lysine into protein at this time is identical at both 32° C. and 37.5° C. As a result, it was decided to more closely investigate the effect of shorter incubation intervals on protein synthesis of the hamster testis at the two temperatures. The results show a very interesting phenomenon. It was observed that at a 7.5 minute incubation, the incorporation of radioactive lysine into testicular protein of the hamster is greater at 37.5° C. than it is at 32° C. At a 15 minute incubation, the lysine incorporation into protein is almost identical at both temperatures. Finally, after 60minutes of incubation the rate of protein synthesis in the hamster testis is seen to be greater at 32° C. than it is at 37.5° C. Thus, it appears that the time of incubation is a critical factor for the <u>in vitro</u> determination of the effect

of temperature on testicular protein labeling. It has been stated by numerous investigators (18) that although the true initial velocity of a reaction increases steadily as the temperature is raised, the amount of substrate transformed at any finite time first rises and then falls, giving an apparent optimum temperature. Furthermore, this optimum temperature is not constant, but falls as the time intervalincreases.

Inasmuch as the rate of testicular protein synthesis in the rat appeared to be heat-sensitive, it was decided to determine the temperatures of the peritoneal cavity and the scrotum of the rat as well as the temperature gradient existing between them. It was thought that this apparent heat lability of the testicular protein-synthesizing system of the rat could possibly be compared to the deleterious effects of high temperatures on spermatogenesis as seen by many investigators (1, 10, 14, 25, 50, 52, 67, 80). The results showed that the average abdominal temperature was 37.4 °C. as compared to 34.3 °C. for the scrotum. Thus, there was an approximate 3 °C. temperature difference between the two. These results substantiated the values reported by other investigators (54).

The next line of investigation was to make a comparative study of the intraperitoneal temperatures with the scrotal temperatures of the various mammalian species being investigated. The mean abdominal-scrotal temperature differences for the six species ranged from 3.1 to 4.4 °C. with the intraperitoneal temperatures always higher than those of the testis. These values are close to those of Wislocki (78) but differ from those of Harrison and Weiner (34) who found the abdominal-testicular temperature gradient for the rat, mouse, and rabbit to be 8.3, 8.4, and 6.2 °C. respectively. One

possible explanation for these differences could be that in Harrison's study, the room temperature was 65° F. or 18.3° C., far lower than the 25 °C. room temperature used in the present study. Moore and Quick (54) have showed that the difference between peritoneal and scrotal temperatures increases with decreasing environmental temperature. In any event, a definite difference has been found between the abdominal and scrotal temperatures of the six mammals in question.

The next objective was to find the reason why the abdominal-scrotal temperature gradients were much larger in some animals than in others. Among the factors involved in producing these temperature gradients are the size, shape, position, coverings (11) and possible intrinsic heat production of the testis as well as the possible role of the dartos muscle. Harrison and Weiner (32) have stated that some features of the vascular architecture such as the testicular blood flow, the tortuosity of the testicular artery, and its relation to the pampiniform venous plexus appear to provide a likely mechanism for ensuing this temperature gradient. In fact, remarkable differences in the vascular pattern of the mammalian species under investigation has already been demonstrated. Glover (26) thought it important to establish the range of temperature in which testes situated in a scrotum normally function, for he believed that the peculiar vascular pattern of the mammalian testis indicated that efficient thermoregulation of the testis was vital.

The next line of investigation was to attempt to relate the normal intraperitoneal and testicular temperatures of the particular species to its testicular protein-synthesizing system and its subsequent dependence on

temperature. It will be remembered that in the rat, mouse, and hamster, the incorporation of L-lysine-U-C^{14} into protein of testis slices was greater at 32°C. than it was at 37.5°C. It will also be remembered that the normal testicular temperatures of the rat and hamster and scrotal temperature of the mouse were 33.4, 34.8, and 33.7°C. respectively. The intraperitoneal temperatures of the rat, mouse, and hamster were 37.7, 38.1, and 37.9°C. respectively. These results, therefore, reveal that protein biosynthesis in the testis of the rat, mouse, and hamster, was maximal at a temperature close to that of the corresponding testicular temperature (32°C) and that the lysine incorporation into testicular protein was adversely affected by a temperature nearly equivalent to the normal intra-abdominal temperature of these three species (37.5°C). The rate of protein biosynthesis was shown to be lower at 26°C. than at 32°C.

The same type of relationship can be shown for the rabbit, guinea pig, and dog who all showed a greater incorporation of labeled lysine into testicular protein at 37.5°C. than at 32°C. It has also been shown in this study that virtually no radioactivity was found to be incorporated into protein at 44°C. In addition, Hall (28) showed a sharp decline in protein biosynthesis in the rabbit when the incubation temperature was increased from 38° to 40°C. Thus, there appears to be an apparent optimal temperature of 37.5°C. The normal testicular temperatures of the rabbit, guinea pig, and dog were shown to be 36.9, 36.4, and 35.0°C. respectively, whereas the intraperitoneal temperatures were 40.0, 39.8, and 38.5°C. respectively. It should be remembered that the dog was anesthetized and, as a result, the testicular and abdominal temperatures reported here could actually be

lower than normal. Thus, these results also reveal that the incorporation of L-lysine-U-C^{14} into testicular protein of the rabbit, guinea pig, and dog was maximal at a temperature closely approximating the normal testicular temperature (37.5 °C) and that protein biosynthesis was adversely affected by a temperature nearly equivalent to the intra-abdominal temperature of the three species (40 °C). Thus, this apparent difference between the first group of species (rat, mouse, and hamster) which incorporated labeled lysine maximally into testicular protein at 32 °C. and the second group of species (rabbit, guinea pig, and dog) which shows maximal rate of testicular protein biosynthesis at 37.5 °C., could possibly be explained by stating that each group of species has its own position on the temperature scale in regard to the optimal temperature for maximal protein biosynthesis. In this study it appears that the critical factors are both testicular and intraperitoneal temperatures. Johnson et al. (38) first noticed this "species specific optimal temperature phenomenon" when studying the influence of temperature on the luminescence intensity of different species of bacteria as a unique measure of enzyme action within the living cell. He concluded that any factor whose influence on luminescence is affected by temperature must be considered in relation to the temperature characteristics of the particular species involved. He thought that the recognition of a reversible denaturation is a major factor in the temperature-velocity relation. The different optimal temperatures for various species indicates that the reversible denaturation reaction becomes appreciable in each species at a different critical temperature; high for some, lower for others.

It was, therefore, seen in each of the six species studied that testicular

protein biosynthesis was near a minimum at a temperature approximating that of the peritoneal cavity. The question then arose as to the manner in which these higher temperatures affect testicular protein biosynthesis. Fukui (25) believes that the testicular proteins are heat labile at body temperature. Many investigators (63) believe that heat kills by enzyme or protein inactivation. As the temperature rises, enzyme activity increases, and destruction of the enzyme is also accelerated; above a certain temperature the enzyme is so rapidly destroyed that the net effect is reduced activity. Wasteneys and Borsook (77) state that the effect of temperature in vitro is on the equilibrium position between protein and its hydrolyzed products so that an increase of temperature results in a higher equilibrium yield of protein, while a decrease in temperature diminishes the equilibrium yield of protein. In other words, increase in temperature favors synthesis and decrease in temperature favors hydrolysis of protein.

The observation that the normal testicular temperatures of the rabbit, guinea pig, and dog are significantly higher than those of the rat, mouse, and hamster suggests that the testicular enzymes of the first group are more thermo-stable than those of the second group. This would also be indicated by the fact that maximal incorporation of labeled lysine into testicular protein occurred at a higher temperature in the rabbit, guinea pig, and dog than it did in the rat, mouse, and hamster. This difference in the heat lability of the enzymes of the protein-synthesizing systems of the two groups of mammals is thus apparently an important biochemical distinction. Moore (53) has observed a similar physiological difference among certain warm-blooded animals. He states that the testicular function of the bird is normal despite a normal abdominal position of the testes.

As a result, he believes that there exists an innate physiological difference in the two classes of warm-blooded vertebrates exhibited by the thermolabile character of the mammalian testis and a more thermostable one in the bird. Wislocki (78) also believes that the testes of various mammalian species differ in their response to body temperature inasmuch as some species such as Monotremata, Proboscidea, and some Insectivora retain their testes permanently in the abdomen.

In further investigating the possible reasons for species differentiation in protein biosynthesis, it was discovered that Balogh and Cohen (2) had made a comparative study of the oxidative enzyme systems of epididymal spermatozoa of adult male mice, rats, guinea pigs, and dogs. He found that there were no major differences in the enzyme activity of these species although each species did appear to have its own predominant enzyme. The mouse showed an excess of DPNH diaphorase; the rat showed a preference for succinic dehydrogenase; the guinea pig seemed to favor lactic dehyrogenase; and, the dog had the greatest concentration of TPNH diaphorase. Other enzymes found in all species were malic, isocitric, and glucose-6-phosphate dehydrogenase. It was also seen that these enzymes were concentrated in the midpieces of the spermatozoa, and that the midpieces varied anatomically from species to species. Rats and mice were seen to have unusually long midpieces as compared to dogs and guinea pigs. The fact that these investigators have shown differences in the predominant enzymes found in various mammalian species could throw light on the possibility that the enzymes needed for protein synthesis might differ in different species, even in regard to their temperature sensitivity characteristics.

Other investigators have also discussed biochemical differences among the various species in question. Elton et al. (21) has observed that ACTH fails to produce a depletion of adrenal ascorbic acid in rabbits and dogs, but does produce a significant depletion of adrenal ascorbic acid in mice and hamsters. Thorsteinsson (69) has shown that the peptidase activity of rabbit semen was due to three separate enzymes. He also saw that more than three-fourths of the peptidase activity toward the substrate glycl-L-leucine was lost upon incubation of the semen for 30 minutes at 38°C. This loss of peptidase activity at 38°C. is the type of phenomenon which could be used to explain the reason for the maximal rate of testicular protein synthesis of the rabbit at 37.5°C. Finally, it was observed by Tobach and Gold (70) that guinea pigs when put in a brightly-lit arena differed in their response from other rodents such as rats, mice, and hamsters.

Munro (55) has observed that the body size of different animals affects their metabolic activities, expecially those dealing with protein metabolism. He states that the total energy expenditure per kilogram of body weight is less in large animals. As a result, large animals do not need to consume so much food per unit of body weight and, therefore, the uptake of protein and nutrients in general decline in relation to weight. As a consequence of the diminishing protein intake, many aspects of protein metabolism become less intense as the size of the animal increases. It might be anticipated that this effect of body size on the intensity of protein synthesis would be reflected in differences in the concentration of nucleic acids in the tissues, since the nucleic acids are concerned with protein synthesis. Munro and Downie (56) found that as the size of different species of mammals increases, the amount of RNA per hepatic cell progressively decreases. Along with

this was a reduction in the intensity of plasma albumin synthesis by larger animals. Begg et al. (5) has shown that with increasing size of mammals, there is also a diminution in the concentration of RNA and, to a lesser extent of DNA and phospholipid in the thyroid gland. It is thus likely that the turnover of thyroid protein is slower in larger mammals.

The relationship of these findings to the present study that protein synthesis is stimulated by an increase in temperature in the larger mammals (rabbit, guinea pig, and dog) and decreased by an elevated temperature in the smaller mammals (rat, mouse, and hamster) is not completely clear, although at first glance it does appear contrary to the results of Munro (55) (56). Two questions remain unanswered. First of all, how does the size of the animal affect the RNA content of the testis? Secondly, what is the effect of temperature on the concentration of testicular nucleic acids?

The next consideration was to determine the sexual maturity of the testis of the six species under investigation. This point was of concern because of the work of Davis and co-workers (14) who showed that the incorporation of L-lysine-U-C^{14} into protein of the immature testis of the rat increased with an increase in temperature from 32 to 37.5° C. following a one hour incubation period; these results were contrary to those found for the mature testis of the adult rat. As a result, two criteria were used to determine the maturity of the testis of the various mammals. First, a comparison was made between the onset of puberty and the average age of the animals at the time of experimentation. Secondly, a histological examination of the testis of the rat, mouse, hamster, rabbit, guinea pig, and dog was performed at the time of experimentation. In each case, it was revealed that the testes of the

various animals being studied were sexually mature. It would therefore appear that the differences observed in protein biosynthesis in the testes of these species cannot be attributed to a difference in their sexual maturation level.

Many criteria have been used in this study in an attempt to elucidate the reasons for the apparent species differentiation in influencing the effect of temperature on testicular protein biosynthesis. The fact that vascular differences were found among the various mammals (such as the tortuosity and calibre of the testicular artery and its relationship to the pampiniform venous plexus) would tend to explain the temperature gradients found between the abdomen and scrotum. Yet this would not seem to be a satisfactory explanation for the species differences found in testicular protein labeling. Two criteria which were found to be similar in all the species were the position of the testes in the scrotum and the morphological appearance of the testis as seen by histological examination. These two findings would seem to indicate a species similarity rather than a species difference. As a result, it can be speculated that there may be biochemical differences in the protein synthesizing systems of one group of species (rat, mouse, and hamster) as compared to the other (rabbit, guinea pig, and dog). One fact which substantiates this hypothesis is that the enzymes involved in protein synthesis of the rat, mouse, and hamster are apparently more heat labile than those of the rabbit, guinea pig, and dog. However, in addition to this enzymatic sensitivity to temperature, other biochemical parameters dealing with the metabolic formation of testicular protein and which might indicate a difference in species activity should be investigated in future studies. Among

these are the effect of temperature on testicular nucleic acid synthesis (DNA and RNA) and the effect of temperature on the testicular energy generating systems (ATP).

CHAPTER V
SUMMARY

1. The aerobic incorporation of L-lysine-U-C^{14} into testicular protein of various mammalian species has been studied following a one hour incubation period in vitro. It was observed that in the testis of the rat, mouse, and hamster, maximal incorporation of labeled lysine into protein occurred at 32° C. However, in the testis of the rabbit, guinea pig, and dog, protein labeling occurred maximally at a temperature of 37.5°C.
2. A comparison of the peritoneal with the scrotal temperatures of the adult male rat, mouse, hamster, rabbit, guinea pig, and dog demonstrated that in each case, the testicular temperature was three to four degrees lower than the corresponding abdominal temperature.
3. The abdominal temperatures of the rabbit, guinea pig, and dog were in general statistically higher than the abdominal temperatures of the rat, mouse, and hamster.
4. The scrotal temperatures of the rabbit, guinea pig, and dog were in general statistically higher than the scrotal temperatures of the rat, mouse, and hamster.
5. Testicular protein biosynthesis in the sexually mature rat, mouse, hamster, rabbit, guinea pig, and dog appears to be maximal at an incubation temperature which most closely approximates their corresponding testicular temperatures.
6. Testicular protein biosynthesis in the sexually mature rat, mouse, hamster, rabbit, guinea pig, and dog appears to be adversely affected at an incubation temperature which most closely approximates their

normal abdominal temperatures.

7. These data indicate that the protein synthesizing mechanisms involved in the incorporation of labeled lysine into protein of the testis of the rat, mouse, and hamster are more heat labile than those of the rabbit, guinea pig, and dog.

8. The finding that there appears to be a difference in heat sensitivity of the protein synthesizing systems of the rat, mouse, and hamster as compared to the rabbit, guinea pig, and dog indicates a basic biochemical difference in spermatogenesis between these two groups of species of common laboratory animals.

CHAPTER VI

CHARTS, TABLES, AND FIGURES

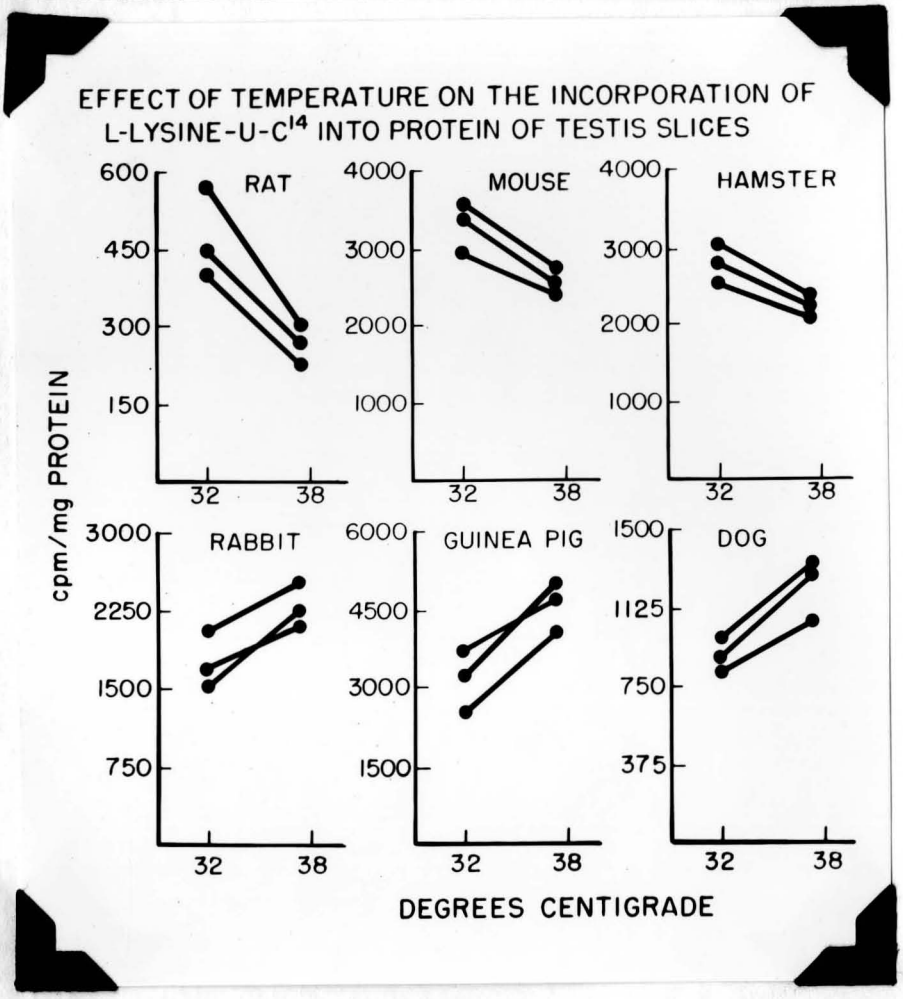

CHART 1

Effect of temperature on the incorporation of L-lysine-U-C^{14} into protein of testicular slices of the adult rat, mouse, hamster, rabbit, guinea pig, and dog. Each line represents the tissue obtained from a single animal. The values are expressed as counts per minute per milligram protein following a one hour incubation period at 32° and 37.5°C.

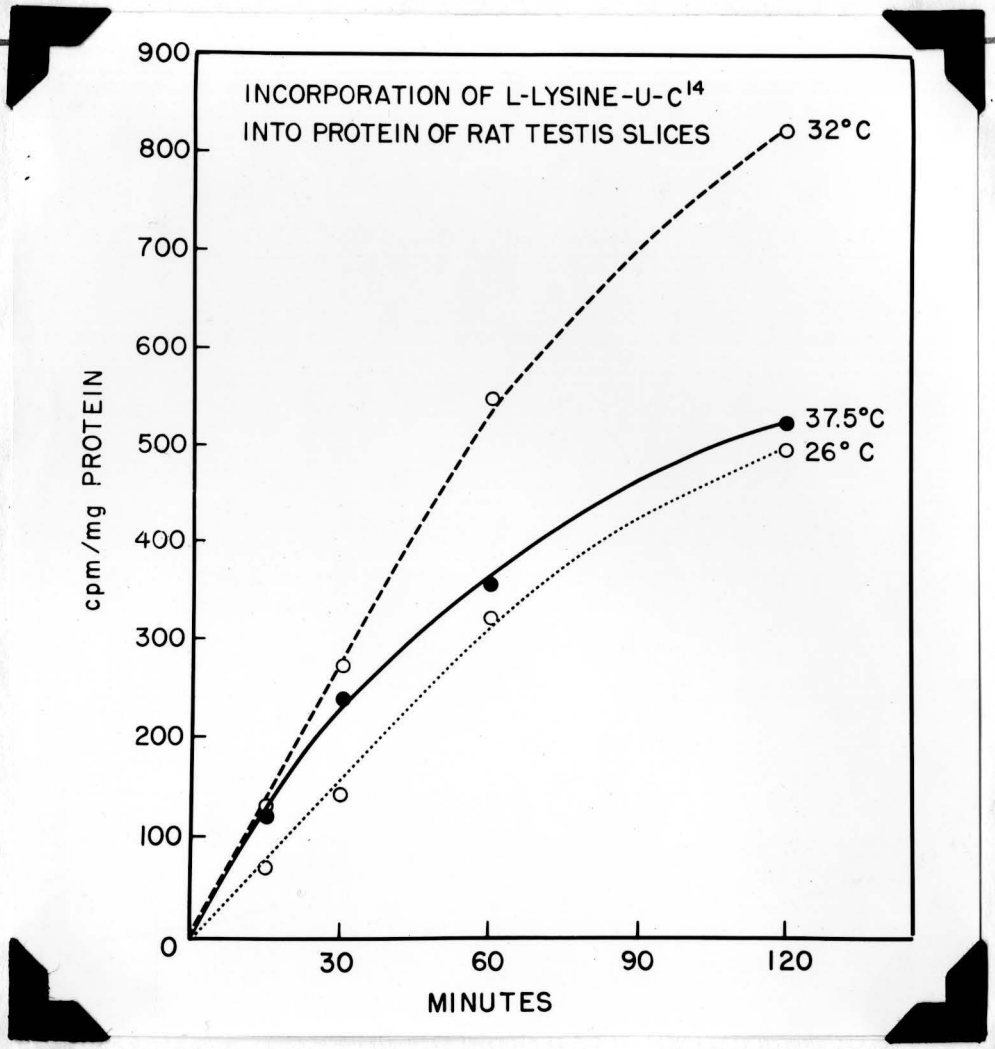

CHART 2

Incorporation of L-lysine-U-C^{14} into protein of rat testis slices at 26°, 32°, and 37.5°C following various incubation times. Flasks containing tissue samples from the same animal were run concurrently thus eliminating a great deal of biological variation. The abscissa is the incubation time in terms of minutes while the ordinate is the specific activity expressed as counts per minute per milligram dry protein. Each point on the curves represents the average of three experiments.

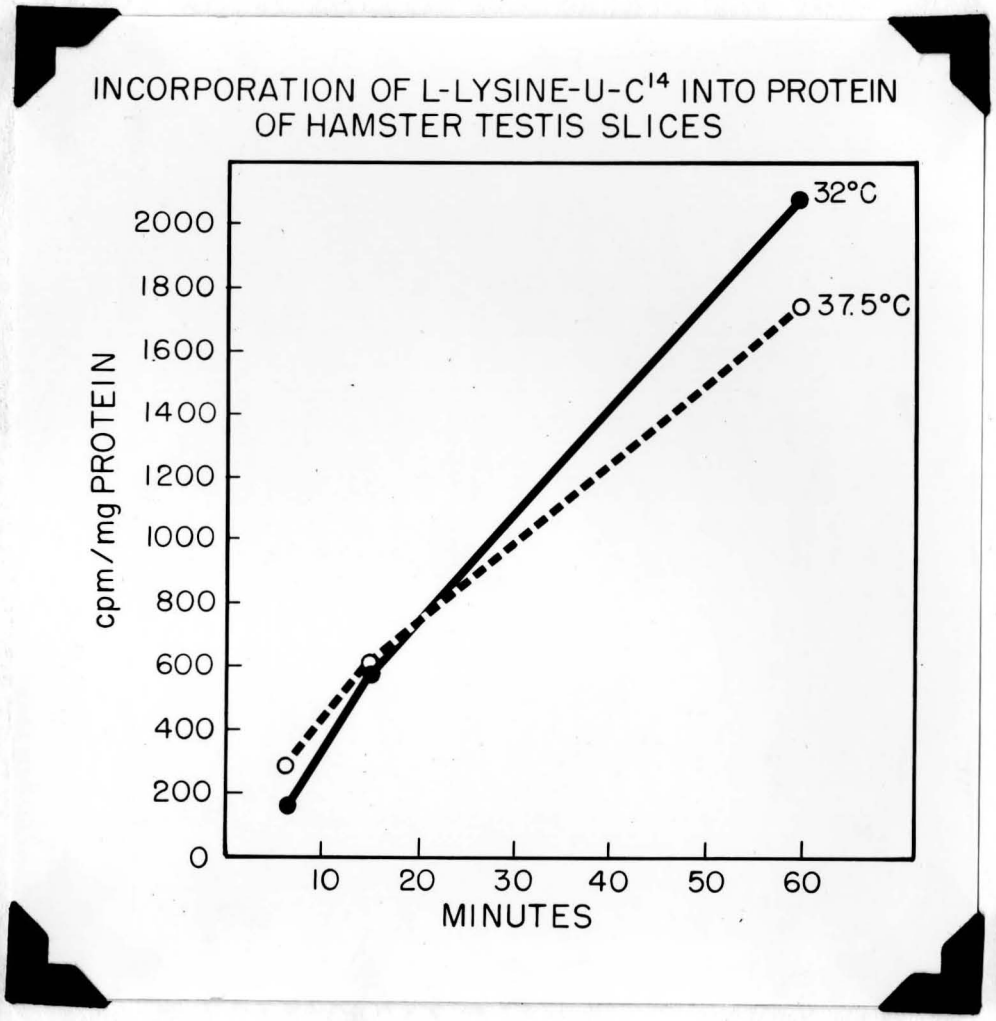

CHART 3

Kinetic study of the incorporation of L-lysine-U-C^{14} into protein of hamster testis slices at 32° and 37.5° C. at various times of incubation. Each point on the curves represents the average of four experiments.

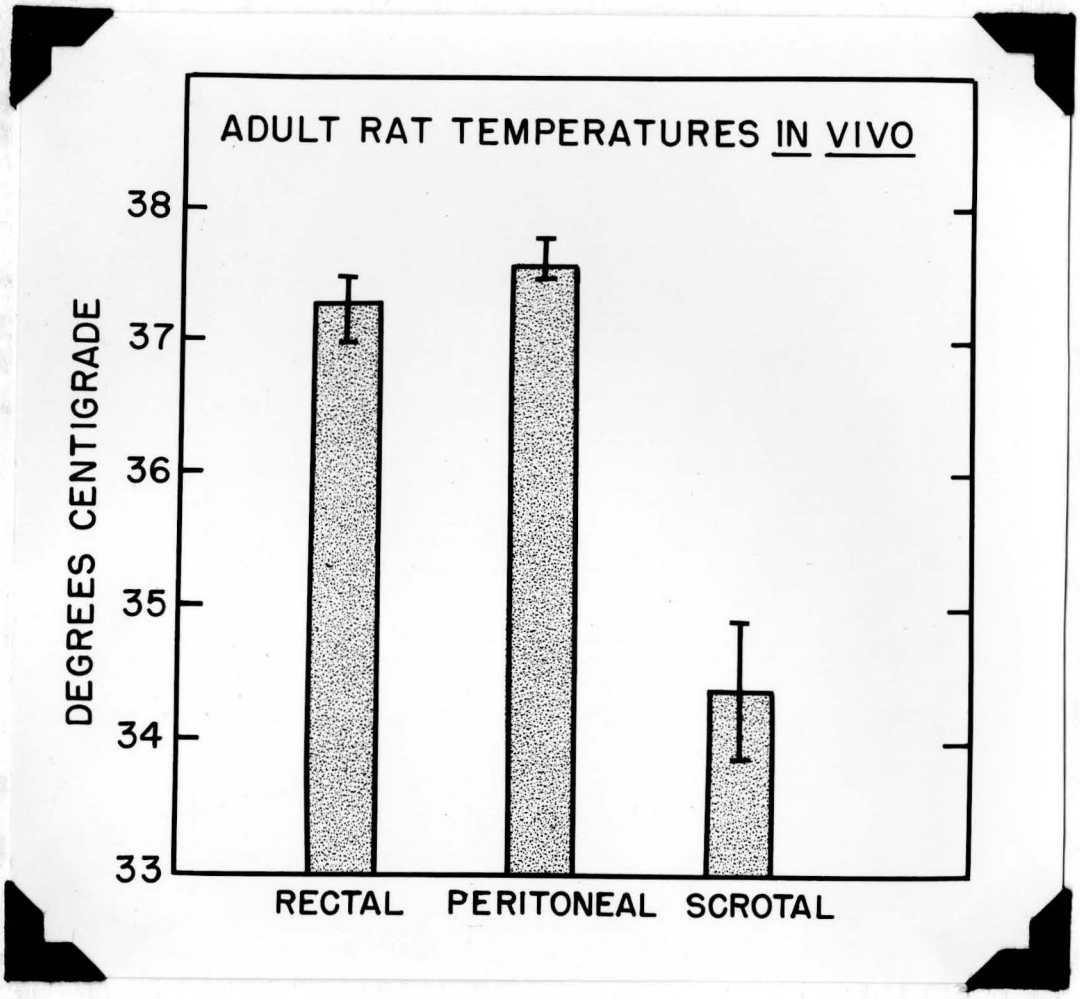

CHART 4

In vivo temperature measurements of the rectal, peritoneal, and scrotal regions of the adult male rat. Recordings were obtained by utilizing thermocouple probes at room temperature. Each bar represents the average of five animals. The vertical bars represent the range.

TABLE I

COMPARISON OF INTRAPERITONEAL WITH SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS

ANIMAL NO.	RAT		MOUSE		HAMSTER	
	I.P.	SCROTAL	I.P.	SCROTAL	I.P.	SCROTAL
1.	37.2	33.3	37.1	33.0	37.8	35.2
2.	38.1	33.2	37.6	33.6	37.8	34.4
3.	37.0	33.4	38.5	34.4	38.0	34.7
4.	37.8	33.6	38.6	34.4	38.0	34.9
5.	38.2	33.1	38.6	33.2	38.0	34.7
Mean[1]	37.7	33.4	38.1	33.7	37.9	34.8
S.E.	0.24	0.09	0.31	0.29	0.05	0.13
P.	<0.001		<0.001		<0.001	

I Expressed in degree centigrade

TABLE I (con't)

COMPARISON OF INTRAPERITONEAL WITH SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS[1]

ANIMAL NO.	RABBIT		GUINEA PIG		DOG	
	I.P.	SCROTAL	I.P.	SCROTAL	I.P.	SCROTAL
1.	39.5	36.2	40.4	36.2	38.2	35.3
2.	40.2	36.7	39.8	36.2	38.5	34.7
3.	39.7	36.5	39.7	36.2	38.6	35.3
4.	39.9	37.7	39.8	36.6	38.0	34.4
5.	40.7	37.6	39.4	36.9	39.2	35.4
Mean[2]	40.0	36.9	39.8	36.4	38.5	35.0
S.E.	0.21	0.30	0.16	0.15	0.20	0.22
P	<0.001		<0.001		<0.001	

1 All dogs were anesthetized with 50 mg./kg. of pentothal sodium injected intramuscularly.

2 Expressed in degrees centigrade

TABLE II

COMPARATIVE STUDY OF BOTH INTRAPERITONEAL AND SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS[1]

INTRAPERITONEAL TEMPERATURE		
Animal	Degrees Centigrade	Standard Error
Rat	37.7	± 0.24
Mouse	38.1	± 0.31
Hamster	37.9	± 0.05
Rabbit	40.0	± 0.21
Guinea Pig	39.8	± 0.16
Dog	38.5	± 0.20

SCROTAL TEMPERATURE		
Animal	Degrees Centigrade	Standard Error
Rat	33.4	± 0.09
Mouse	33.7	± 0.29
Hamster	34.8	± 0.13
Rabbit	36.9	± 0.30
Guinea Pig	36.4	± 0.15
Dog	35.0	± 0.22

1 All animals with the exception of the dog were unanesthetized.

TABLE III

SIGNIFICANCE OF THE DIFFERENCE BETWEEN SCROTAL TEMPERATURES IN UNANESTHETIZED ANIMALS[1]

	Rat	Rabbit	Rat	Guinea Pig	Rat	Dog
Scrotal Temperature (°C.)	33.4	37.6	33.4	36.4	33.4	35.0
Difference (°C.)	4.2		3.0		1.6	
t values	11.06		17.40		7.36	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.001	

	Mouse	Rabbit	Mouse	Guinea Pig	Mouse	Dog
Scrotal Temperature (°C.)	33.7	36.9	33.7	36.4	33.7	35.0
Difference (°C.)	3.2		2.7		1.3	
t values	7.36		8.10		3.64	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.001	

	Hamster	Rabbit	Hamster	Guinea Pig	Hamster	Dog
Scrotal Temperature (°C.)	34.8	36.9	34.8	36.4	34.8	35.0
Difference (°C.)	2.1		1.6		0.2	
t values	6.30		8.16		0.84	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.5	

1 All animals with the exception of the dog were unanesthetized.

TABLE IV

SIGNIFICANCE OF THE DIFFERENCE BETWEEN INTRAPERITONEAL TEMPERATURES IN UNANESTHETIZED ANIMALS[1]

	Rat	Rabbit	Rat	Guinea Pig	Rat	Dog
Mean IP Temperature (°C.)	37.7	40.0	37.7	39.8	37.7	38.5
Difference (°C.)	2.3		2.1		0.8	
t values	7.27		7.14		2.53	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.05	

	Mouse	Rabbit	Mouse	Guinea Pig	Mouse	Dog
Mean IP Temperature (°C.)	38.1	40.0	38.1	39.8	38.1	38.5
Difference (°C.)	1.9		1.7		0.4	
t values	5.02		4.93		1.08	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.5	

	Hamster	Rabbit	Hamster	Guinea Pig	Hamster	Dog
Mean IP Temperature (°C.)	37.9	40.0	37.9	39.8	37.9	38.5
Difference (°C.)	2.1		1.9		0.6	
t values	9.74		11.08		2.84	
D.F.	8		8		8	
P values	<0.001		<0.001		<0.05	

1 All animals with the exception of the dog were unanesthetized.

TABLE V

COMPARISON OF THE ONSET OF PUBERTY IN COMMON LABORATORY ANIMALS

	Strain	Average Weight	Average Age	Approximate Onset of Puberty	Total Life Span
Rat	Sprague-Dawley	220 gms.	60 days	35 days	3-3 1/2 yrs.
Mouse	Swiss-Webster	22 gms.	7 weeks	6 weeks	2-3 yrs.
Hamster	Golden Syrian	85 gms.	8 weeks	6 weeks	2 years
Rabbit	New Zealand White	3.9 kg.	6 months	6 months	2 years
Guinea Pig	Harley	491 gms.	10 weeks	10 weeks	6 years
Dog	Mongrel	9.5 kg.	2-5 years	10-13months	12 years

The average age and average weight were taken at the time of experimentation.

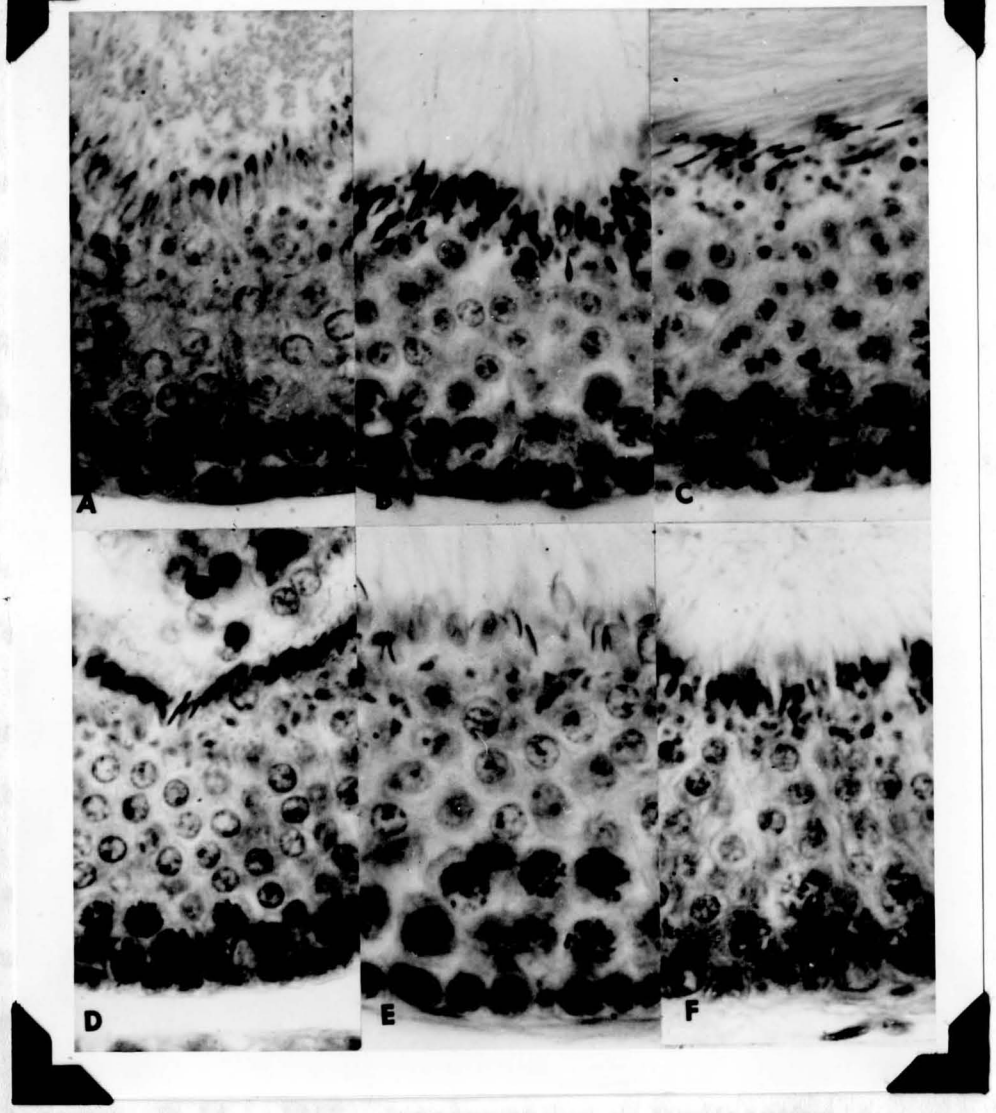

FIGURE I

Representative photomicrographs of sections of the testis of the adult rat (A), mouse (B), hamster (C), rabbit (D), guinea pig (E), and dog (F). The tissues were sectioned at 5 microns and stained with hematoxylin and eosin. Magnification is approximately x 650. The age of the animals employed at both the time of incubation with L-lysine-U-C^{14} and histological fixation were as follows: Rat, 60 days; Mouse, 7 weeks; Hamster, 8 weeks; Rabbit, 6 months; Guinea Pig, 10 weeks; and Dog, 2-5 years.

CHAPTER VII

BIBLIOGRAPHY

1. Asdell, S.A. and Salisbury 1941 The Rate at Which Spermatogenesis Occurs in the Rabbit. Anatomical Record, 80, 145-151.

2. Balogh, K. and Cohen, R.B. 1964 A Cytochemical Technique for studying Oxidative Enzyme Systems of Mammalian Spermatozoa in Semen Smears. Fertility and Sterility 15, No. 1, 35-39.

3. Barron, D.H. 1933 The Trophic Influence of the Spermatic Nerve on the Testicle. Anatomical Record, 55, 6.

4. Bartlett, D.J. 1962 Studies on Dog Semen II. Biochemical Characteristics. Journal of Reproduction and Fertility, 3, 190-205.

5. Begg, D.J., McGirr, E.M., and Munro, H.N., 1965 Protein and Nucleic Acid Content of the Thyroid Glands of Different Mammals. Endocrinology, 76, No. 2, 171-177.

6. Bhargava, P.M. 1957 Incorporation of Radioactive Amino Acids in the Protein of Bull Spermatozoa. Nature, 179, 1120-1121.

7. Bishop, M.W.H. and Walton, A. 1960 Spermatogenesis and the Structure of Mammalian Spermatozoa. Chap. 7 in Marshall"s Physiology of Reproduction edit. by Parkes, A.S. Vol. 1, Part 2, 20-24.

8. Bishop, M.W.H. and Walton, A. 1960 Metabolism and Motility

of Mammalian Spermatozoa. Chap. 9A in Marshall's Physiology Reproduction edit. by Parkes, A.S. Vol. 1, Part 2, 264-280.

9. Borsook H. 1949 Uptake of Labeled Amino Acids by Tissue Proteins in Vitro. Federation Proceedings, 8, 589-596.

10. Chowdhury, A.K. and Steinberger, E. 1964 A Quantitative Study of the Effect of Heat on Germinal Epithelium of Rat Testes. American Journal of Anatomy, 115, No. 3, 809-524.

11. Crew, F.A.E. 1922 A Suggestion as to the Cause of the Aspermatic Condition of the Imperfectly Descended Testis. Journal of Anatomy, 56, 98-106.

12. Cross, B.A. and Silver I.A. 1962 Neurovascular Control of Oxygen Tension in the Testis and Epididymis. Journal of Reproduction and Fertility, 3, 377-395.

13. Dahl, E.V. and Herrick, J.F. 1959 A Vascular Mechanism for Maintaining Testicular Temperature by Counter-Current Exchange. Surgery, Gynecology, and Obstetrics, 108, 697-705.

14. Davis, J.R., Morris, R.N. and Hollinger, M.A. 1964 Incorporation of L-lysine-U-C^{14} into Proteins of Crytorchid Testis Slices. American Journal of Physiology, 207, No. 1, 50-54.

15. Davis, J.R., Firlit, C. F., and Hollinger, M.A. 1963 Effect of Temperature on Incorporation of L-lysine-U-C^{14} into Testicular Proteins. American Journal of Physiology, 204, No. 4 696-698.

16. Davis, J.R. and Morris, R.N. 1963 Effect of Glucose on Incorporation of L-lysine-U-C^{14} into Testicular Proteins. American Journal of Physiology, 205, No. 5, 833-836.

17. Dickens, F. and Greville, G.D. 1933 Metabolism of Normal and Tumor Tissue VIII. Respiration in Fructose and in Sugar-Free Media. Biochemical Journal, 27, Part 5, 832-841.

18. Dixon, M. and Webb, E.C. 1964 Effect of Temperature on Enzyme Kinetics. In Enzymes edit. by Dixon and Webb, 2nd edition, 145-150.

19. Eckstein, P. and Zuckerman, S. 1956 Morphology of the Reproductive Tract. Marshall's Physiology of Reproduction edit. by Parkes, A.S. Vol. 1, Part 1, 43-155.

20. Elliott, K.A.C., Greig, M.E., and Benoy, M.P. 1937 The Metabolism of Lactic and Pyruvic Acids in Normal and Tumor Tissue III. Rat Liver, Brain, and Testis. Biochemical Journal, 31, Part 2, 1003-1020.

21. Elton, R.L., Zarrow, I.G., and Zarrow, M.X. 1959 Depletion of Adrenal Ascorbic Acid and Cholesterol. A Comparative Study. Endocrinology, 65, 152-157.

22. Ewing, L.L. and Vandermark, N.L. 1963 Factors Affecting Testicular Metabolism and Function. II. Effect of Temperature Elevation in vivo on Subsequent Metabolic Activity of Rabbit Testicular Tissue in vitro. Journal of Reproduction and Fertility, 6, 9-16.

23. Ewing, L.L. and Vandermark, N.L. 1963 Factors Affecting Testicular Metabolism and Function. III. Effect of In vitro Temperature Elevation on Tissue Slices and Perfused Testes in the Rabbit. Journal of Reproduction and Fertility, 6, 17-24.

24. Flipse, R.J. 1956 Metabolism of Glycine by Bovine Spermatozoa. Science, 124, 228.

25. Fukui, N. 1923 On a Hitherto Unknown Action of Heat Ray on Testicles. Japan Medical World, 3, 26-28.

26. Glover, T.D. and Young, D.H. 1963 Temperature and the Production of Spermatozoa. Fertility and Sterility, 14, 441-450.

27. Gospodarowicz, D. and Legault-Demare 1962 Influence de la temperature d'incubation sur la synthese in vitro de certains steroids dans le testicule de Rat. Acadamie Des Sciences Comptes Rendus Des Seances, 255, Part 3, 3047-3049.

28. Hall, P.F. 1965 Influence of Temperature upon the Biosynthesis of Testosterone by Rabbit Testis In Vitro. Endocrinology, 76, No. 3, 396-402.

29. Hall, P.F. and Eik-nes, K.B. 1962 The Action of Gonadotropic Hormones Upon Rabbit Testis In Vitro. Biochimica et Biophysica Acta. 63, 411-422.

30. Hardy, S.M. 1961 Amino-Acid Liberation from Proteins by Enzyme or Heat Catalyzed Hydrolysis. Nature, 191, 1061-1065.

31. Harrison, R.G. 1956 Factors influencing the Process of Spermatogenesis in the Experimental Animal. British Journal of Urology, 28, 422-425.

32. Harrison, R.G. and Weiner, J.S. 1949 Vascular Patterns of the Mammalian Testis and their Functional Significance. Journal of Experimental Biology, 26, 304-317.

33. Harrison, R.G. 1949 The Distribution of the Vasal and Cremasteric Arteries to the Testis and their Functional Importance. Journal of Anatomy, 83, 267-317.

34. Harrison, R.G. and Weiner, J.S. 1948 Abdomino-testicular Temperature Gradients. Journal of Physiology, 107, 48P-49P.

35. Herrington, L.P. and Nelbach, J.H. 1942 Relation of Gland Weights to Growth and Aging Processes in Rats exposed to Certain Environmental Conditions. Endocrinology, 30, 375-386.

36. Hill, R.T. 1937 Ovaries Secrete Male Hormone. Endocrinology, 21, 495-502.

37. Hoffman, R.A., Hester, R.J., and Towns, C.M. 1964 Effect of Light and Temperature on the Endocrine System of the Golden Hamster. American Zoologist, 4, No. 3, 296.

38. Johnson, F.H., Brown, D., and Marsland, D. 1942 A Basic Mechanism in the Biological Effects of Temperature, Pressure, and Narcotics. Science, 95, 200-203.

39. Kauzmann, W. 1954 Denaturation of Proteins and Enzymes. In The Mechanism of Enzyme Action edit. by McElroy, W.D. and Glass, B., 70-121.

40. Lardy, H.A. and Ghosh, D. 1949 A New Metabolic Regulator in Mammalian Spermatozoa. Federation Proceedings, 8, 218.

41. Leblond, C.P. and Clermont, Y. 1952 Spermiogenesis of Rat, Mouse Hamster, and Guinea Pig as Revealed by the "Periodic Acid-Fuchsin Sulfurous Acid" Technique. American Journal of Anatomy, 90, 167-206.

42. Lundquist, F. 1950 Liberation of Amino Acids in Human Seminal Fluid. Federation Proceedings, 9, 198.

43. Lundquist, F. 1951 Studies on the Biochemistry of Human Semen. IV. Amino Acids and Proteolytic Enzymes. Acta Physiologica Scandinavia, 25, 178-187.

44. Macdonald, J. and Harrison, R.G. 1954 Effect of Low Temperature on Rat Spermatogenesis. Fertility and Sterility, 5, 205-216.

45. Mann, T. and Lutwak-Mann, C. 1948 Studies on the Metabolism of Semen. IV. Aerobic and Anaerobic Utilization of Fructose by Spermatozoa and Seminal Vesicles. Biochemical Journal, 43, 266-270.

46. Mann, T. and White, I.G. 1957 Glycerol Metabolism by Spermatozoa. Biochemical Journal, 65, 634-639.

47. Mann, T. and Parsons, U. 1947 Effect of Testicular Hormone on the Formation of Seminal Fructose. Nature, 160, 294.

48. Martin, F. and Brachet, J. 1959 Autoradiographic Studies on the Incorporation of Amino Acids into Spermatozoa. Experimental Cell Research, 17, 399-404.

49. Moore, C.R. 1924-25 Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. VI. Testicular Reactions in Experimental Cryptorchidism. American Journal of Anatomy, 34, 269-317.

50. Moore, C.R. and Chase, H.D. 1923 Heat Application and Testicular Degeneration. Anatomical Record, 26, 344-345.

51. Moore, C.R. 1926 The Activity of Displaced Testes and its Bearing on the Problem of the Function of the Scrotum. American Journal of Physiology, 77, 59-68.

52. Moore, C.R. and Oslund, R. 1923 Experimental Studies on Sheep Testes. Anatomical Record, 26, 343-344.

53. Moore, C.R. 1924-25 Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. VIII. Heat Application and Testicular Degeneration; The Function of the Scrotum. American Journal of Anatomy, 34, 337-358.

54. Moore, C.R. and Quick, W.J. 1923 A Comparison of Scrotal and Peritoneal Temperatures. Anatomical Record, 26, 344.

55. Munro, H.N. 1964 General Aspects of the Regulation of Protein Metabolism by Diet and by Hormones. In Mammalian Protein Metabolism edit. by Munro, H.N. and Allison, J.B. 1, 381-481.

56. Munro, H.N. and Downie, E.D. 1964 Relationship of Liver Composition to Intensity of Protein Metabolism in Different Mammals. Nature, 203, No. 4945, 603-604.

57. Neame, K.D. 1962 Uptake of L-Histidine, L-Proline, L-Tyrosine, and L-Ornithnine by Brain, Intestinal Mucose, Testis, Kidney, Spleen, Liver, Heart Muscle, Skeletal Muscle, and Erythrocytes of the Rat in vitro. Journal of Physiology, 162, 1-12.

58. Nelson, W.O. 1951 Mammalian Spermatogenesis: Effect of Experimental Cryptorchidism in the Rat and Non-Descent of the Testis in Man. Recent Progress Hormone Research, 6, 29-62.

59. Niemi, M. and Ikonen, M. 1962 Cytochemistry of Oxidative Enzyme Systems in the Leydig Cells of the Rat Testis and their Functional Significance. Endocrinology, 70, 167-174.

60. Nimni, M.E. and Geiger, E. 1957 Testosterone-Induced N-Retention in Castrated Rats on Diets Containing Incomplete Proteins. Endocrinology, 61, 753-757.

61. Oakberg, E.F. 1956 A Description of Spermiogenesis in the Mouse and its Use in Analysis of the Cycle of the Seminiferous Epithelium and Germ Cell Renewal. Amer. J. Anat. 99,391-

62. Prosser, C.L. 1964 Comparative Physiology and Biochemistry Status and Prospects. Comparative Biochemistry and Physiology, Vol. 11, No. 1, 1-7.

63. Prosser, C.L. 1950 Temperature: Metabolic Aspects and Perception. In Comparative Animal Physiology edit. by Prosser, C.L. 341-380.

64. Roy, A. and Bishop, M.W.H. 1954 Effect of Glycine on the Survival of Bull Spermatozoa in vitro. Nature, 174, 746-747.

65. Scheraga, H.A. 1963 Intramolecular Bonds in Proteins. II. Noncovalent Bonds. In The Proteins edit. by Neurath, H. Second edition, Vol. 1, 477-594.

66. Sizer, I.W. 1943 The Inactivation of Enzymes by Heat. In Advances in Enzymology edit. by Nord, F.F. and Werkman, C.H. 3, 41-45.

67. Steinberger, E. and Dixon, W.J. 1959 Some Obeervations on the Effect of Heat on the Testicular Germinal Epithelium. Fertility and Sterility, 10, 578-595.

68. Tepperman, J., Tepperman, H.M., and Dick, H.J. 1949 A Study of the Metabolism of Rat Testis in Vitro. Endocrinology, 45, 491-503.

69. Thorsteinsson, T. 1958 Proteolytic Activity of Human, Rabbit, and Bull Semen with Special Reference to Peptidases in the Genital Tract of Male Rabbit. American Journal of Physiology, 194, 341-343.

70. Tobach, E. and Gold, P.S. 1964 The Response of Guinea Pigs to an "Unfamiliar Environment". American Zoologist, 4, No. 3, 274 (abstract).

71. Tosic, J. 1947 Mechanism of Hydrogen Peroxide Formation by Spermatozoa and the Role of Amino Acids in Sperm Motility. Nature, Lond., 159, 544

72. Turner, C.D. 1960 Endocrinology of the Testis. In General Endocrinology by Turner, C.D., 331-364.

73. Turpeinen, P., Turpeinen, D., and Talanti, S. 1962 Effect of Local Heat in Vivo on Hyaluronidase, Succinic Dehydrogenase, and Phosphatases of the Rat Testis. Endocrinology, 70, 731-737.

74. Ulberg, L.C. 1958 The Influence of High Temperature on Reproduction. Journal of Heredity, 49, 62-62.

75. Waites, G.M.H. and Moule, G.R. 1961 Relation of Vascular Heat Exchange to Temperature Regulation in the Testis of the Ram. Journal of Reproduction and Fertility, 2, 213-224.

76. Walton, A. 1930 The Effect of Temperature on the Survival In Vitro of Rabbit Spermatozoa Obtained from the Vas Deferens. Journal of Experimental Biology, 7, 201-219.

77. Wasteneys, H. and Borsook, H. 1930 The Enzymatic Synthesis of Protein. Physiological Reviews, 10, 110-145.

78. White, A., Handler, P., Smith, E., and Stetten, D. 1959 Enzymes. In Principles of Biochemistry, Second Edition, 232-257.

79. Wislocki, G.B. 1933 Location of the Testes and Body Temperature in Mammals. Quarterly Review of Biology, 8, 385-396.

80. Wolf, R.C. and Leathem, J.H. 1955 Hormonal and Nutritional Influence on the Biochemical Composition of the Rat Testis. Endocrinology, 57, 286-290.

81. Young, W.C. 1927 The Influence of High Temperature on the Guinea Pig Testis. Journal of Experimental Zoology, 48-49, 459-499.

APPROVAL SHEET

The thesis submitted by Richard Buyer has been read and approved by three members of the faculty of Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 21, 1965
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