THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report
of
Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given David Maurice Siperstein final oral examination for the degree of Master of Arts . We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 24, 1910

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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee
of the Graduate School, have read the accompanying
thesis submitted by David Maurice Siperstein
for the degree of Master of Arts.

They approve it as a thesis meeting the require-
ments of the Graduate School of the University of
Minnesota, and recommend that it be accepted in
partial fulfillment of the requirements for the
degree of Master of Arts.

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The Effects of Acute and Chronic Inanition upon the
Development and Structure of the Testis in the
Albino Rat.

A thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota

by

David M. Siperstein

In partial fulfillment of the requirements
For the degree of
Master of Arts.

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The Effects of Acute and Chronic Inanition upon the
Development and Structure of the Testis in the Albino Rat.

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I. Introduction.

The immediate and the remote effects of inanition upon the young organism are subjects of interest and importance, especially at the present time. Experimental studies in this field have yielded much of importance to biology in general and to medicine in particular. It is very desirable to know more about the changes produced in the sex glands of malnourished or undernourished infants and children, and the resulting effects upon the reproductive system in later adult life. Inanition in adults may also occur either alone or associated with acute or especially ^{with} chronic wasting diseases, causing degenerative processes in the sex glands. Recent work on vitamine deficiencies (by McCarrison, Allen, Dutcher and others) indicates that the changes produced in the sex glands by this partial inanition resemble those found after general inanition. A study of the changes occurring in the histological structure of the testis of the albino rat during experimental inanition at various ages was therefore undertaken, and the results are embodied in the present paper.

II. Literature.

Falck ('54) was apparently the first to mention the effects of inanition on the testis. He found the relative (percentage) weight of the testis in dogs to be unaffected by inanition.

On the other hand, Voit ('66) stated that, as a result of inanition, there is a relative decrease in the weight of the testis.

Manassein ('68) concluded that inanition causes but slight change in the relative weight of the testis.

Morpurgo ('88) ('89) investigated the effects of inanition

upon cell division in young and adult rabbits. He found mitoses in general to continue, though reduced in number, in various organs during inanition. He observed mitotic figures in the testis of an adult rabbit that died on the 13th day of acute starvation, and concluded that inanition does not markedly affect the process of cell-division in that organ. As stated by him: "Unter den hoch differenzirten Organen sindes ausschliesslich die Geschlechtsdrüsen, welche während der Inanition und trotz des Inanitionszustandes des Organismus einen lebhaften Zelltheilungsprocess zeigen."

Grandis ('89) observed marked atrophy of the testis in fasting pigeons. Formation of spermatozoa always ceases within twelve days, although mitosis continues in the seminiferous epithelium. He found that the spermatids and spermatozoa already formed die and are resorbed. As inanition proceeds, (with loss of over 40 per cent in body weight), the greater part of the seminal epithelium is progressively involved. Grandis thought that this degenerating material furnishes nutrition to the starving animal. The parietal cells of the tubules persist and, upon refeeding, these cells regenerate the tubules. The cells of the seminal epithelium assume an embryonic appearance during inanition. He found the normal dimensions of the testis (1 x 2 cm) to be reduced to one-half or less by 17 days of total inanition. In two cases, which were starved for 24 days, with loss of 51.5 and 54.5 per cent in body weight, the diameter of the seminiferous tubules was reduced to 10-15 μ (1/10 to 3/20 normal), and the seminal epithelium was reduced to two indistinct cell layers. The fibrous trabeculae also become extremely atrophied.

In 1895, v. Hansemann reported that in hibernating marmots there is no evidence of spermatogenesis. However, after two months recovery from the winter's sleep, spermatogenesis becomes normal and the interstitial substance becomes filled with so many cells as to give the impression of a large cell sarcoma. However, it should be noted that the changes taking place in the organs of the body during hibernation may be entirely distinct from those changes that occur as a result of ordinary inanition. It must also be remembered that hibernating animals have a normal seasonal sexual cycle, which is independent of the nutritive conditions.

Pernice and Scagliosi ('95) studied the effect of thirst (water-inanition) on the testes of young chickens. They described the germinal cells as very much atrophied. Spermatozoa are not present in the lumina of the tubules. There is also a total cessation of karyokinesis in the spermatogonia. The thickened tunica albuginea becomes infiltrated with small lymphocytes, and the testes appear hyperemic.

Simonowitsch ('96) subjected rabbits and guinea pigs to total inanition. He emphasized the phase of the sexual cycle of the testis, which must be considered in interpreting the microscopic structure; for the cells of the testis normally present differences according to the stage of spermatogenesis. He observed that acute inanition causes a parenchymatous degeneration (cloudy swelling and granular degeneration) accompanied by fatty degeneration, vacuolization of cells and chromatolysis. The interstitial tissue exhibits an edematous infiltration. However, this condition occurs only irregularly among the tubules. Most of the tubules remain apparently normal. Upon refeeding, the

testis returns to normal in less than a week, and the interstitial tissue becomes markedly infiltrated with fat. Chronic inanition produces more marked degeneration, and one that appears more evenly distributed throughout the organ.

Miescher (¹⁸⁹⁰~~1897~~) studied salmon during the spawning period. As is well known, during the ascent up the river the salmon do not feed. Miescher found that during this period the entire musculature of the body becomes atrophied, while the sex glands increase greatly in size and develop normally. In other words, the gonads develop at the expense of the rest of the body.

Cordes ('98) described the conditions found in the human testis following acute and chronic diseases. In chronic conditions involving inanition and cachexia, there is invariably an increased amount of interstitial tissue, together with a thickening of the walls of the seminal tubules. In a series of 21 cases of chronic disease, there was a total lack of spermatogenesis in 16. He was never able to demonstrate any change in the fat content of the seminal epithelium, in either acute or chronic disease.

De Bruyne ('99) claimed that the polynucleated cells found in the seminal epithelium of rats are precursors of degeneration.

Gaule ('01) came to the conclusion that in the frog the other organs of the body furnish material to develop the gonads. As proof he pointed to the fact "dass bei der Esculenta ja die Laichzeit im Beginn der Fressperiode liegt, dass also das Material welches zur Reifung der Eier gebraucht wird, aus dem Körper aus anderer Organen stammen muss."

In the same year, Loisel ('01) reported that in a dog kept alive for 26 days on a water diet, spermatogenesis was arrested in most of the tubules. There was a marked tendency towards a

reduction of the seminal epithelium to a single layer of cells, the cells of Sertoli. He believed that the cells of Sertoli retain the power to regenerate sperm-cells, in case the animal is refed.

Ganfini ('03) claimed that Hansemann's conclusion, that in hibernating marmots the interstitial cells are reduced in number, is not warranted. Instead, he said, there is only a decrease in the size of the cells and some alteration in their structure. (As above mentioned, it must be remembered that hibernation may produce effects entirely distinct from those during true inanition.)

Traina ('04) found that even with a loss of body weight of 20 to 25 per cent, adult rabbits show no decrease in spermatogenesis. Only after a loss in body weight of 30 to 35 per cent does spermatogenesis cease. But even here, karyokinetic figures are always to be found among the spermatocytes and spermatogonia. As to fat content he concluded: "Die Hodenepithelien enthalten unter normalen Verhältnissen eine reichliche Fettmenge, welche mit dem Alter zunimmt und in keinem Zusammenhang mit den Funktionszuständen steht. Dieser Fettgehalt bleibt bei Marantischen und Hungerzuständen unverändert."

Nussbaum ('06) studied the effects of acute inanition in the testes of *Rana fusca*. He found that on the 21st of June, the testes of freshly caught frogs had the following measurements: length, 9 mm.; width, 5 mm.; thickness, 3 mm. On the 29th of July, after a continuous period of starvation dating from the 21st of June, the testes measured: length, 16 mm.; width, 10 mm.; thickness, 7 mm. While in June the testes contained only a few

spermatogonia, in July, in spite of the inanition, the process had developed to the spermatid stage. Microscopically, the testes were normal in structure and in the proper degree of development. He came to the conclusion that "die Geschlechtsorgane wachsen auf Kosten der Fette und Muskeln des Körpers."

Gerhartz ('09) also studied the effects of starvation on *Rana fusca*. He used two series of frogs; one series fed on frog's legs, the other subjected to complete inanition from the 19th of September to the 10th of December. The results were as follows. In series 1 (control) the testis averaged 9.25 mm. long, 6.11 mm. wide, 5.14 mm. thick. The average body weight was 36.9 grams. The average volume of the testis was 179.8 cu.mm. He concluded: "Die Produkte der Hodenmasse als Ausdruck ihrer Grosse genommen, verhalten sich fast genau Zueinander wie die Körpergewichte, so dass die Abnahme der Hoden proportional der Abnahme des Körpergewichtes ging."

Kyrle ('10) summarized the results of ^{his} histological studies on 220 testes of individuals who had died between birth and eighteen years of age. He described the normal testis of the newborn as having the tubules closely packed together, with very little interstitial tissue. The membrana propria has a cylindrical epithelium. A distinct lumen is present in the center of the tubules. In cases with malnutrition and retarded development, the tubules become somewhat shrunken in size, and the lumina disappear. The interstitial connective tissue increases in relative amount and hemorrhages were observed therein.

Monterosso ('12) studied the effects of inanition (total and water only) on the rat and mouse. He always obtained a cellular degeneration, proceeding in inverse order to that of spermatogenesis. The Sertoli cells resist the longest, but after 200 hours of acute inanition (in the rat), their nuclei also disappear. Degeneration is preceded by the formation of polynucleated giant cells, which in turn degenerate by a process of karyolysis. The lipoid in the interstitial tissue cells is entirely independent of the lipoid in the seminal epithelium, since in the interstitial tissue the lipoid material disappears during inanition, while persisting in the seminal epithelium.

Voss ('13) worked on malnourished children and confirmed the results obtained by Kyrle ('10). He stated that "die Angabe Kyrles, dass nur ein kleiner Prozentsatz der kindlichen Hoden eine gute Entwicklung der Samenkanälchen aufweist, und dass die überwiegende Mehrzahl bei geringer Entwicklung der Kanälchen eine starke Entwicklung des interstitiellen Gewebes zeigt, wird durch meine Kontrolluntersuchungen vollauf bestätigt."

Poiarkoff ('13) subjected two dogs for three months to a diet restricted to 1/4 lb. of oats in meat bouillon. The body weight of one dog decreased from 46 to 31 pounds; that of the other from 32 to 21 pounds. Both dogs were then refed and recovered their initial weight in two months. During the period of inanition, he removed one testis from one of the dogs (does not say which dog) for microscopic study, and found a decrease in the activity of spermatogenesis. Many tubules contained only Sertoli cells; others had only a few spermatocytes. Spermatogonia were rare, and all the tubules showed marked degenerative changes. On refeeding, he

found that both the seminal fluid and the number of spermatozoa increased in amount and returned gradually to normal. His findings led him to believe that a "castration caused by inanition" is possible.

Jackson ('15) concluded that "during inanition the loss in weight of both testis and epididymis is relatively not very different from that in the body as a whole, and is (like that of the majority of the viscera) more marked in chronic than in acute inanition". He found that as a result of a_cute inanition, the testes of the adult albino rat decreased from an average of 2.50 to 1.756 grams (a loss of 29.8 per cent); while as a result of chronic inanition, the decline was from 2.27 to 1.355 grams (a loss of 40.3 per cent). Later ('15a) he reported that in young rats held at constant body weight by underfeeding, beginning at 3 weeks of age, there was found an increase in the weight of the testes.

Hatai ('15) used a lipoid-free diet consisting of casein 18 per cent, lactose 20 per cent, agar 2 per cent, dextrin 56 per cent and various salts. He found that in the male albino rat on this diet the testes were considerably (44 per cent) smaller than in the normal control. He therefore concluded that the testes not only had failed to grow during nearly six months of the special diet, but that there is a clear indication of an actual loss in weight.

Morgulis, Howe and Hawk ('15) studied the tissues of fasting dogs with varying losses in body weight (up to 50 per cent). They found a total absence of karyokinetic figures in the testis. They stated that "in a very large proportion of the tubules the chromatin substance of all the nuclei was massed together to one

side, as in the case of synizesis".

Mann ('16) stated that during hibernation the sex glands of gophers apparently undergo no specific changes due to the torpid condition. This, however, may have little relation to the condition produced by a true inanition.

In 1916, Stewart found that in the albino rat held at maintenance from 3 to 12 weeks of age the testis is constantly below normal in weight, especially towards the end of the maintenance period. On refeeding, the testis may remain subnormal in weight for a short time, but later becomes normal.

Swingle ('18) reported that total starvation of *Rana pipiens* larvae inhibits their growth and metamorphosis, Spermatogenesis ceases and the onset of sex differentiation is prevented.

Stewart ('18) showed that while the body of albino rats is held at birth weight by underfeeding for 16 days, the testes apparently gain an average of 374 per cent in weight. In those underfed for longer periods (with increasing body weight) the gain is smaller, being 188, 51 and 34 per cent, respectively. In the test rats underfed for very long periods, beginning at three weeks of age and continuing for about a year, the testes were found 42 per cent subnormal in weight.

Rasmussen ('18) found that in the woodchuck (*Marmota monax*), spermatogenesis remains stationary during the torpid state. The interstitial tissue shows no appreciable change, other than a slight decrease in the amount of pigmentation. (As previously mentioned, however, the state of hibernation is quite different from that of ordinary inanition.)

Miles ('19) used a low protein diet on a squad of 12 men, using a similar normally fed squad as a control. The "test squad" showed a decrease in body weight of 12 per cent. A marked decrease in sexual desire was noted among these men.

In 1919, Jackson and Stewart published further results on the refeeding of young albino rats that had been underfed from birth for various periods. It was found that the relative overweight of the testes previously found at the end of the underfeeding period persists to a certain extent in the rats refed to a body weight of 25 to 50 grams. When the rats were refed to 75 grams, however, there is a marked deficit (-44.6 to -49.4 per cent) in the weight of the testis. It was suggested that the earlier period of inanition, while permitting immediate growth of the testis upon refeeding, might affect it in some way so as to inhibit its later growth and development.

McCarrison ('19) experimented with a vitamine-free (polished rice) diet on pigeons, which produces a polyneuritis (beri-beri) with atrophic changes in various organs, especially the sex glands. In the healthy adult pigeon, the average weight of the testes per kilo of body weight was 4.643 grams; but in the test pigeons only 0.4015 grams--a reduction to 1/11 of the original weight. Thus a profound atrophy of the testis occurs. The interstitial tissue has increased, but the tubules appear greatly contracted and the seminiferous epithelium has almost completely disappeared.

Allen ('19) investigated the effect on albino rats of a diet deficient in water-soluble vitamins. It results in a complete degeneration of the seminiferous cells. The Sertoli cells appear the most resistant. There is a hypertrophy of the interstitial tissue.

Dutcher ('20) studied the effect of a polished rice diet on cockerels. After three to five weeks, the testis has greatly atrophied, often being reduced to one-fifth of the normal in both size and weight. He and Wilkins found that by this diet they could get a marked atrophy of the testis without any loss of body weight. This, according to Dutcher, "eliminates any possibility of explaining the condition on a starvation or inanition basis". Upon removing one testis and adding green alfalfa to the rice diet, the remaining testis increased in size and weight so as to approach the normal.

Jackson and Stewart ('20) reported that when underfed young albino rats are fully refed to maximum body weight, the testes are definitely above normal (+9 to +30 per cent) for the corresponding body weight.

III. Material and Methods.

A large part of the material for this investigation was presented to me by Dr. C. M. Jackson, to whom the writer is also very much indebted for constant advice and criticism throughout the course of this study. The material comprises the testes of several albino rats (*Mus norvegicus albinus*) that had been subjected to inanition experiments in the Institute of Anatomy. The adult animals during acute inanition were allowed water only; but one of the adults was placed on total inanition, as were also the young rats (2 days old) which were removed from the mother and given neither food nor water. Those on chronic inanition were fed restricted amounts of Graham bread soaked in whole milk for various periods. Some were refed fully after underfeeding for various periods. The animals were killed by chloroform and autopsied, the testes being weighed (without epididymis) and portions fixed for histological study. For convenience the material at my disposal is grouped as shown in table 1. In the first column, representing the individual rat numbers, the letters indicate the series (those autopsied by myself are "S1"), the number following indicates the litter, while the number after the decimal point identifies the individual rat.

The testes had been fixed in either Zenker's, Bouin's or Flemming's fixatives. Paraffin sections were cut at 2 to 10 micra in thickness. The sections were stained chiefly with haematoxylin and eosin, Weigert's iron haematoxylin counterstained with Van Gieson's stain, or Heidenhain's iron-alum haematoxylin counterstained in some instances with orange G or acid fuchsin. In general, Heidenhain's iron-alum haematoxylin without any counterstain gave by far the best results.

My own material was fixed in Carnoy's mixture (No. 1) and in Bouin's fluid. Fixation for one hour with Carnoy's mixture was found to be best. Sections were stained with Heidenhain's iron-alum haematoxylin with or without counterstain (acid fuchsin). This gave clear cut karyokinetic figures in all stages of mitosis.

IV. Observations.

1. Normal Postnatal Histogenesis (group I).

In order to understand more fully the changes which inanition produces in the testis, it is essential to review briefly its normal histological structure and histogenesis, as a basis for comparison.

Newborn (Fig. 1). In microscopic sections of the testis in the newborn rat, the seminiferous tubules appear closely packed together at the periphery of the testis, while in the center of the section the tubules are less compactly arranged. The inter-tubular spaces contain many small capillary vessels and mesenchymal cells. The tubules themselves consist chiefly of a single layer of parietal epithelial cells, which apparently correspond to those designated as "Sertoli cells" by v. Ebner ('02) in the testis of the colt. These cells fuse to form a syncytium. Lumina have not yet appeared in the tubules. The central portion of each tubule contains from one to three large clear cells, each containing a definite, well-marked nucleus. Each nucleus has from one to three small clumps of chromatin situated usually (but not always) close to the periphery of the nucleus. These masses probably represent chromatin nucleoli. These large central cells apparently correspond to those described by v. Ebner ('02, fig. 1147) as the primitive germ cells ("Ursamenzellen") from which the spermatogonia are probably derived.

Interstitial cells (of typical structure) have not yet developed. There is present, however, in the intertubular tissue a peculiar type of cell which appears to be the forerunner of the later interstitial cells. This cell contains a large round nucleus extremely rich in chromatin which in turn is surrounded by a narrow

rim of clear cytoplasm. By the form of its nucleus and its deeply staining chromatin, it is easily distinguished from the ordinary young mesenchymal or connective tissue cell.

Fourth day (Fig. 2). In a normal rat at four days of age the development of the testis has advanced considerably. The whole section is filled with seminiferous tubules. Within the tubules themselves, the parietal cells show no material increase in number, but the spermatogonia (Allen's cell Type A) have appeared. They are probably derived from the central cells ("Ursamenzellen" of v. Ebner), which still occur (less numerous) and present mitoses, though less frequently than in the newborn. Before dividing, they often appear to migrate from the center of the tubule toward its periphery and crowd in among the parietal cells. Some tubules already contain a few cells which resemble Allen's type B,--that is, a primary spermatocyte cell in its earliest phase. The forerunners of the interstitial cells are beginning to group themselves together in the intertubular spaces.

One week (Fig. 3). A section of the testis from an albino rat at seven days shows an increase in the number of tubules. This represents the earliest stage described by Allen ('18). The tubules now contain two or three layers of cells. Very few central cells still remain, but spermatogonia (Allen's type A) are present in large numbers. There are very few type B cells. In other words, an undifferentiated condition of the spermatogenic epithelium still prevails. The syncytium (Sertoli syncytium) which fills the tubules at this stage is exceptionally well shown. The number of mitotic figures has again increased, so there are now as many figures in the field as there were at birth. The

interstitial cells are more numerous and the amount of interstitial tissue is increased.

Two weeks (Fig. 4). There is a large increase in the number of seminiferous tubules, but no change in their size. There are many cells of types A and B, and some tubules even show Allen's type C (spermatocytes in which the chromatin masses become more diffuse than in type B and assume a "woolly appearance"). A few cells have advanced as far as the leptotene stage. It is now fairly easy to distinguish between Sertoli cells and spermatogonia. The Sertoli cell has a nucleus with only a little chromatin material, in this way differing from the nucleus of the spermatogonium, which has a large amount of deeply staining chromatin.

Three weeks (Fig. 5). Lumina are just beginning to appear in the closely packed tubules. Spermatogonia, cells of types A, B, and C are numerous. A few tubules already contain primary spermatocytes showing mitotic figures. In many places, degenerating primary spermatocytes and spermatogonia are also present. This appears to be a normal physiological process, occurring in the testis at all ages. No secondary spermatocytes or spermatids are to be seen. The interstitial tissue is assuming its adult structure.

One month (Fig. 6). The testis at this stage shows only a slight advance in development. It is distinguished by the large number of primary spermatocytes present. Spermatids have not yet appeared. The interstitial tissue shows no change.

Six to ten weeks. Sections of testes from the 43 to 70 days of age show a gradual increase in the size of the tubules and in the number of mature sperm elements. Mature spermatozoa appear

for the first time at the age of 43 days. Their number increases so rapidly that at eight weeks (Fig. 7) the histological structure of the testis differs in no respect from that in the older rats. The interstitial cells show no noticeable change in structure.

Judging from the statements of Hewer ('14) and Allen ('18), there appears to be considerable variation in the age at which the various steps in the process of spermatogenesis occur in the albino rat. Hewer reported that there is no differentiation of primary spermatocytes from spermatogonia until the 25th day after birth. Allen found that his rats reached the pachytene stage at two weeks; while my material first shows this stage at three weeks. Hewer found that lumina first appear in the tubules at 7 weeks. Allen demonstrated them in all rats 14 days old. In my own rats 14 days old, I found no evidence of definite lumina, but they begin to appear at 21 days. Hewer stated that spermatozoa are not present in the lumina of the tubules until nine weeks after birth. Allen found spermatozoa in rats at the age of 37 days, which corresponds closely with my observation at 43 days.

2. Changes in young rats subjected to acute inanition. (Group II)

A comparison of the testes of the normal control (at four days) with those of the test animals of same age (but lesser body weight) in table 1 shows but little apparent retardation in the growth in weight of the gonads of the latter (average weight in two controls, 0.0053 grams; in three test rats, 0.0046 grams). Although the test rats were kept on total inanition for 48-50 hours, the testes have evidently maintained nearly normal growth, although the body weight has decreased 21 to 25 per cent.

The remarkably persistent growth of the testes in the starved rats is especially evident upon comparison with the newborn of the same litter (Si 1.4). The starved rats have decreased in body weight until they, although four days old, are about 20 per cent below the normal newborn. The testes, however, show a large increase in weight (in spite of the total inanition). The change in weight from .0029 to an average of .0046 grams represents an increase of 59 per cent. This is in general agreement with the results of Stewart ('18), who found that in rats kept at maintenance (constant body weight) by underfeeding from birth to (average) 16 days the testis apparently increased about 374 per cent in weight. The smaller increase in the testes of my starved rats is probably due to the greater severity and shorter duration of the inanition.

All three of the starved young rats (Si 1.1, Si 2.3, Si 2.6) show practically the same histological structure in the testis. The tubules are close together and more numerous than in the newborn, although there are relatively few in the center of the section. Cell differentiation has not progressed much beyond that found in the newborn. Each tubule presents the single layer of parietal cells. These cells are all alike and contain large nuclei, with very little chromatin material, surrounded by a narrow strip of clear cytoplasm. The central cells ("Ursamenzellen" of v. Ebner) like those already described in the normal testis are also still present. There are very few mitotic figures either among the parietal cells or the central cells (Fig. 8). Sections (stained with iron-alum haematoxylin) of the testes of controls show many mitotic figures in each field

(Figs. 1 and 2). This decrease in the number of karyokinetic figures is probably due to the condition of inanition. In general, the histological picture shows that the testis has advanced to the stage when type A cells are beginning to appear. Thus the stage of differentiation is somewhat beyond that corresponding to the normal at similar body weight, but behind that corresponding to the age or actual testis weight. The increase in the number of tubules probably accounts for the increase in the size (weight) of the testis.

3. Changes in rats held at maintenance by underfeeding from three to ten weeks of age (Group III).

In the five rats of this group held nearly at constant body weight by underfeeding from three to about ten weeks of age, the testes, though below normal weight for that age, have been retarded in their growth far less than has the body weight. Thus on comparing the average data for group III (table 1) with the normal control at three weeks (S1 6.0) it appears that the body weight has increased from 20.6 gm. to 26.9 gm., an increase of about 31 per cent; while the testes have increased from 0.920 gm. to an average of .1956 gm., an increase of about 113 per cent. This would indicate a relative growth for the testis even greater than that (+34 per cent) found by Jackson ('15a) under similar conditions. Individual variations in the control or test rats probably account for the difference.

With the exception of one rat (S 11.65), which will be mentioned later, the testes in this group all present the same microscopic picture (Fig. 9). The tubules are placed close together. Lumina are just beginning to appear although most of the

tubules still show no central lumen. No spermatozoa or spermatids are present. There is no evidence to indicate that they had ever been developed and had undergone necrosis. A few secondary spermatocytes can be seen, but they are abnormal in structure.

Almost every primary spermatocyte also shows some degenerative changes. The cytoplasm becomes coarsely granular and contains clumps of basophilic staining material. Other cells show a fatty (?) degeneration or vacuolization of the cytoplasm (a positive statement cannot be made as material stained for fat was not available). The nuclei degenerating by a process of karyorrhexis may give rise to the basophilic granules present in the cytoplasm of so many spermatocytes. The pyknotic nuclei are very abundant and, with progressive necrobiosis, appear gradually to lose their staining capacity terminating in karyolysis. The cytoplasm also progressively loses its staining capacity and gradually disappears.

The spermatogonia, however, appear normal. The Sertoli cells are increased in number, but show no structural changes. The tubules are filled with a structure resembling the "degeneration reticulum" described by Kuntz ('20) in the testis after division of the nerves. This appears to me to be due largely to the Sertoli syncytium which becomes visible in direct ratio to the number of other cells which are being destroyed. Allen ('19) pointed out that in normal tubules the Sertoli syncytium is hard to see on account of the closely packed germ cells. As a result of experimental conditions, the syncytium is made more prominent by the removal of the germ cells which normally obscure it.

There are present in all cases many multinucleated or giant cells. These cells are situated in the center of the lumen and

each tubule usually presents one or two of them. Each cell may contain from two to four nuclei, all showing some degenerative changes. Multinucleated cells appear to be very characteristic of the degenerative process in the testis. To a certain extent, the degree of severity of the starvation can be measured by the size of these cells and the number of nuclei they contain. For example, absolute inanition for 9 days (in the adult) produces giant cells containing as high as 12 to 15 nuclei, while in the slower chronic inanition in the present group, the number is reduced to 3 or 4 nuclei.

One other important fact, which is strikingly illustrated in these sections, is the remarkable persistence of mitotic figures in the spermatogonia and primary spermatocytes. In rats which have been kept at constant body weight by underfeeding for seven weeks, the number and structure of the mitotic figures is apparently normal as compared with control rats (from 3 to 4 weeks). This fact is in agreement with the observations by many investigators (Traina, Grandis, Morpurgo, Cordes and others) who have reported the presence of mitotic figures after prolonged chronic inanition. This mitosis is doubtless correlated with the persistent growth of the testis in the underfed young rats under these conditions, the new cells formed being in excess of those destroyed by degeneration.

My study of this group of albino rats kept at maintenance by underfeeding from three to ten weeks leads to the following conclusions: First, the chronic inanition prevents the testis from reaching the stage of spermatogenesis normal for the corresponding age. Second, in spite of the persistent mitosis

and increased size of the testis, the process of spermatogenesis remains at the stage where spermatocytes are produced; corresponding to the normal stage at the age of 3 or 4 weeks. The growth capacity of the testis under these conditions of subnormal nutrition is apparently sufficient only to produce primary spermatocytes from the spermatogonia. Third, the spermatocytes degenerate and are absorbed, though not so rapidly as they are formed. The excess of production over destruction probably accounts for the continued growth in weight of the testis. Fourth, the interstitial tissue is apparently unchanged and resembles in quantity and structure the tissue present in a normal rat at the age of 3 or 4 weeks.

In one rat (S 11.65, Fig. 10) in this group, the testis shows such remarkable changes that it seems advisable to describe it separately. This was the left testis (indicated by (L) in table 1), which was markedly atrophic, weighing only .0496 grams. All the tubules show degenerative processes. Many contain no cells whatever. The lumina are filled with the characteristic "degeneration reticulum", and some have large vacuoles in their centers. Pyknosis, karyorrhexis, and karyolysis are prevalent.

A few of the seminiferous tubules in this case show a type of degeneration which differs entirely from any other thus far described. All the nuclei in every cell in the tubule gradually and almost uniformly lose their basophilic staining capacity and become acidophilic, taking the red counterstains. At the same time the cytoplasm of all the cells fuses together and becomes homogeneous in appearance. So that the entire cytoplasm in the tubules finally becomes one single mass, resembling very closely

the homogeneous mass of necrotic tissue seen in an infant^{ne}. This mass apparently undergoes a process of autolysis, and vacuoles later replace the cytoplasmic substance. This process continues until finally the structure disintegrates and the position of the tubule is indicated by only the membrana propria. In spite of this necrosis in the tubules, the intertubular tissue shows no marked degenerative changes. However, a very irregularly distributed moderate hyperplasia of the interstitial cells is apparent. While not the typical picture produced by the chronic inanition in group III, this condition evidently corresponds to the advanced degenerative process in an extreme case.

4. Changes in young rats retarded by underfeeding for long periods beginning at three weeks of age
(Group IV).

In the three rats underfed from three weeks to 223-428 days of age (table 1) the body has approximately doubled in weight, whereas normally it should have increased to about 12 times the initial weight. The testes are likewise of course far below norm for the corresponding age, though apparently considerably above the norm for the younger rats of similar body weight (rats Si 10 and Si 11; also Donaldson's Wistar norm). Stewart ('18), however, concluded that in rats underfed beginning at 3 weeks of age so as to reach only about 50 grams in body weight at 412 days of age the testes average 42 per cent below normal (for corresponding body weight). It is evident that the changes in the weight of the testis are extremely variable, so it is unsafe to draw general conclusions from only a few cases.

Microscopic sections (stained with haematoxylin and eosin) of the testis show that the size of the tubules in these rats long

underfed has considerably decreased. The number of tubules has also decreased and they have become widely separated (Fig. 11), due to the accumulation of fluid in the interstitial spaces (edematous infiltration.). No mature spermatozoa or spermatids appear, although they are present in normal rats of corresponding body or testis weight. Spermatogenesis here (as in group III) has apparently not passed the primary spermatocyte stage. Most of the tubules contain 3 to 4 layers of cells, composed of spermatocytes and spermatogonia. The former show the most profound changes. Some appear as shadows without any nucleus or cytoplasm. Others have pycnotic nuclei and a homogeneous cytoplasm. Multinucleated giant cells are present and the nuclei in these cells show pycnotic changes. Many spermatogonia and all the Sertoli cells appear normal. Mitotic figures are still present. Apparently some vascular changes have taken place. There is a slight sclerosis in the walls of the blood vessels and also (apparently) a reduced blood supply to the tubules. There is not any noticeable increase in the quantity of interstitial tissue (proper) or any appreciable change in the structure of the interstitial cells; but the quantity of interstitial fluid has enormously increased.

Since all three rats (St 44.1, St 44.4 and St 44.7) show the same histological structure the question of individual variation may be excluded. So that it can be assumed that the "growth tendency" so strongly developed in the testis, is confined to the formation of the earlier cells in the spermatogenetic cycle. That is, the process can go only to the spermatocyte stage. This is exactly what happened in the case of the rats in group III, as before mentioned.

In one rat (M 29.109) inanition was started at 10 weeks of age after the testis had presumably reached the stage when mature spermatozoa are produced. Even though kept at maintenance (constant body weight) by extreme underfeeding for 244 days, this testis shows the following histological structure. Most of the tubules are normal and show well-developed spermatozoa. However, many lumina are filled with a debris made up almost entirely of heads and tails of spermatozoa. A few tubules show a suggestion of a desquamation process, but this is not distributed evenly among the tubules and is probably of no importance. Aside from this the structure of the testis is apparently normal. Mitotic figures are present and all the various stages of spermatogenesis can be followed out. The conditions in this case emphasize the importance of the age factor. It indicates that if the testis has reached sexual maturity before experimental inanition, the effect upon spermatogenesis is much less marked than in cases where the underfeeding is begun at an earlier period, although the further growth of the testis (in weight) may be hindered. No final conclusions can be drawn from this one rat, however, although the results are in agreement with the conclusions of Jackson and Stewart ('20) as to the importance of the age at which the inanition begins.

5. Changes in adult rats after acute inanition (Group V).

As shown in table 1 (group V), adult rats were subjected to acute inanition for periods varying from 9 to 12 days. The loss in body weight varies from 30 to 47 per cent. The testes also apparently lose a great deal in weight, but the exact amount can of course be only roughly estimated. The test rats at the

beginning of the experiment varied in body weight from 216-328 grams. According to the Wistar norm tables compiled by Donaldson ('15), these body weights call for from 2.5 to 2.8 grams of testis. A comparison with the figures in table 1 shows that at autopsy the testes weight stood from 1.468 to 2.43 grams, indicating considerable loss in weight. Jackson ('15) found that the loss in weight of the testis of adult rats during acute inanition is nearly proportional to that of the whole body.

In one rat (Si 9) which was held for 11 days without food or water, the testis had shrunk away from the tunica albuginea and had decreased considerably in size. This was however not apparent upon removing the organ at autopsy, because the intervening space was filled with a fluid, which kept the tunic distended and gave it a normal rounded appearance. This phenomenon has likewise been noted by Allen ('19) in his study of rats subjected to a diet deficient in the water-soluble vitamins.

At first glance, the sections of the testes in this group appear perfectly normal in structure. The majority of the tubules are apparently normal in size and shape and show no signs of degeneration. Spermatogenesis appears to be going on as usual. Spermatozoa are abundant and show no alteration in structure. There is no apparent change in the amount or structure of the interstitial tissue.

However, more careful study shows that in each section, a few tubules have suffered as a result of the inanition. These tubules, though few in number, and placed in immediate relationship with perfectly normal tubules, may show very marked and very severe changes (Fig. 13). The spermatozoa are irregular in structure and show extreme degenerative processes. The spermatids may present

a picture which strongly suggests a true synizesis. This peculiar clumping of the chromatin towards the periphery and towards one side of the nucleus has been described by Monterosso ('12) as a form of degeneration. But I have observed this same structure in almost everyone of my control rats and believe that we are dealing here rather with a rearrangement of the chromatin material in the normal cytomorphosis of the cell.

The secondary and primary spermatocytes show marked necrotic changes. Even the spermatogonia are involved and reveal pyknotic and karyolytic changes in the nuclei. The Sertoli cells are apparently the only ones not severely injured.

Depending on the stage of degeneration, large numbers of multinucleated giant cells are present in the tubules. These cells each contain from 4 or 5 nuclei up to as many as 12 or 15 nuclei. Some tubules are so atrophic and degenerated that only the membrana propria remains, with a few necrotic giant cells in the lumina. These giant cells were carefully studied with special reference to their origin. Apparently they are derived from primary spermatocytes, which fuse together in groups during their desquamation. These cells will be referred to again at a later period.

Desquamation of the epithelial cells in these degenerating tubules is very common and apparently initiates the process of degeneration. This process appears to start with the secondary spermatocytes, but as the sloughing process continues the primary spermatocytes and even the spermatogonia are detached into the degenerating mass in the lumen of the tubule. Degeneration takes place in every case after desquamation has occurred. This

degeneration apparently is also always in inverse order to that of spermatogenesis. The spermatozoa are the first to disappear, then the spermatids, followed by the spermatocytes and spermatogonia. The Sertoli cells are the most resistant. This agrees with the results obtained by Monterosso (in starved rats), Bouin and Garnier (in alcoholized rats), and Regaud and Tournade (after ligation of the spermatic cord). On the other hand Barratt and Arnold ('11) find that the secondary spermatocytes degenerate first and that the spermatids are the most resistant to starvation. Allen ('19) states that (in degeneration due to vitamine deficiency) "the spermatocytes seem to be the first affected, the spermatids next, and the spermatogonia last".

It therefore appears that acute inanition notably affects but comparatively few tubules in the testis, and that the other tubules retain their normal structure. In spite of a loss of about one-third in the weight of the testis, spermatogenesis apparently continues normally in most of the tubules. But the few tubules which for some unexplainable reason are less resistant, show atrophic and necrotic changes which are as marked as any found in chronic inanition. There is no marked atrophy of the other tubules; and no degeneration or decrease in the quantity of the interstitial tissue that would account for this loss in the weight of the testis.

6. Changes in rats refed after underfeeding beginning at three weeks of age and extending for various periods (Group VI).

In one rat (St 18.83) refed for 3-1/2 days (table 1), the refeding period was too short to produce any definite results. There is apparently already some increase in the total body weight, but very little, if any, in the testis. Sections show no definite change from the appearance of the testes in group IV. The primary spermatocytes still show necrotic changes and there is no advance in the stage of spermatogenesis.

In another rat (St 10.86), refed for 14 days after maintenance by underfeeding from 21 to 72 days, many improvements have taken place. The body weight has increased from around 50 to 66.5 gms., but there is no great increase in the weight of the testes. The reason for this becomes more evident upon histological study. Sections (stained with iron-haematoxylin) show no increase in the number or size of the tubules, compared with those of group IV. But all the debris has been removed, and no degenerating cells are to be seen (Fig. 14). No secondary spermatocytes are present. The interstitial cells and their nuclei appear normal (but increased in size). They are, however, definitely increased in number above normal. They are evenly distributed throughout the testis, in this way differing from the condition found by Allen ('19) in the rats of Osborne and Mendel subjected to a diet deficient in the water-soluble vitamins. There are numerous mitotic figures among the spermatocytes and spermatogonia, showing all the stages of the karyokinetic cycle. The blood vessels are distended with blood. It would appear that all the energy in the

testis has been utilized to "clean up" the waste material and to prepare for a further advance. This may account to some extent for the lack of increase in the weight of the testis.

In the rat (S 12.52) which was refed for 37 days, the weight of the testis has greatly increased and histological study shows that spermatogenesis is going on normally. The tubules are normal in structure and contain many mature spermatozoa. The only difference to be found (as compared with normal control testes) is an increase in the amount of interstitial tissue.

We must distinguish between interstitial tissue and interstitial cells. The former term applies to all tissues outside of the tubules proper; the latter (interstitial cells) refers only to the specific cells playing a part in the internal secretion of the testes. In reality, there is no general hypertrophy of the interstitial tissue, but rather a hyperplasia of the interstitial cells proper. There is a marked increase in the number of these cells and a slight increase in their size. They assume a more or less cord-like arrangement. The nuclei of the cells are spherical and rather vesicular, although a few clumps of chromatin are always present close to the periphery. A light granular cytoplasm surrounds the nuclei. After the very young stages (1 to 7 days), mitosis among these cells has never been observed. One possible explanation which may be offered is that mitosis in these cells takes place very rapidly, so that, although cell division occurs, it is rarely found in the sections.

The fourth rat in this group (S 33.118), refed for 206 days, has completely recovered from the effects of the underfeeding and presents a perfectly normal adult histological structure. Even

the interstitial tissue and cells appear normal in quantity and structure.

These results are in accord with the work of Stewart ('15), who found that in rats refed after underfeeding from 3 to 12 weeks of age, the testes remain somewhat below normal weight for a few weeks, but regain normal weight before the adult stage is reached. In later experiments (Stewart '18), in which the underfeeding was prolonged, the testis became markedly subnormal in weight (as found by Jackson '15a). Jackson and Stewart ('19) reported that the testis, which increases in relative weight in young rats during underfeeding, remains somewhat above normal weight during refeeding to a body weight 25 to 50 grams, but is subnormal in those refed to 75 grams body weight. Finally, Jackson and Stewart ('20) found that when the underfed rats are fully refed to maximum body weight, the testes are definitely above normal for the corresponding body weight.

V. Discussion.

1. Origin and significance of polynucleated giant cells.

It has been claimed by some authors that giant cells may occur normally in the testis. I have never seen either a polynucleated or a giant cell in a normal seminiferous tubule. Monterosso ('12) found that these cells are very rare in normal tubules, but occur more frequently during early inanition. He found that their number increases with the length of inanition, and that as many as fifteen nuclei might be seen in a single cell. He believes that they are formed by a fusion of the neighboring cells.

On the other hand, Bouin and Garnier believe that these cells are formed by an abnormal mitosis of the nuclei within the cell. Allen ('19) noticed these cells during the early stages of degeneration, but offered no explanation as to their origin.

My studies lead me to believe that the multinucleated cell is formed by a fusion of primary spermatocyte cells when they begin to degenerate. As a rule these cells show marked nuclear and cytoplasmic degenerative changes later than those cells which break off independently and do not fuse with other cells. This suggests that fusion of cells may be a sort of protective measure from the effects of the inanition. De Bruyne ('99), however, claims that these polynuclear cells are precursors of degeneration. No mitotic figures have ever been observed in any polynucleated giant cell.

2. Mitosis during inanition.

As to the occurrence of mitosis during inanition, my own observations agree with those of Morpurgo ('88)('89), Ganfini ('03), Traina ('04), Barratt and Arnold ('11) and others. Traina, for instance, found that mitotic figures persist after a loss in body weight of 30 to 35 per cent. In adult rats subjected to acute inanition (S 25, S 26, S 27), with loss in body weight of 30, 35, and 39 per cent, mitotic figures are still present in large numbers. Another rat (Si 9), with loss in body weight of 47 per cent, still shows numerous mitotic figures. They are present even in tubules showing extreme degenerative changes. In young rats underfed from the age 3 to 10 weeks, mitosis is apparently unaffected. Even in those animals in which the underfeeding was continued to 400 days or more, the mitotic figures can be seen in many of the tubules. It would therefore appear justifiable to conclude that it is impossible by inanition to suppress mitosis in the epithelium of the seminiferous tubule. The mitoses, however, may be decreased in number, as found in the rats two days old subjected to acute inanition.

The presence or absence of amitosis in the testis has long been a disputed question. Barratt and Arnold ('11) and Monterosso ('12) found amitosis present among degenerating spermatocytes and spermatogonia. Allen ('18)('19) believes that amitosis does not occur. I have likewise never seen a definite case of amitosis in any of my sections.

3. Interstitial tissue during inanition.

Some investigators mention the increased quantity of interstitial tissue, which is occasioned by any degeneration or atrophy of the seminiferous epithelium. Cordes ('98) found an increase in interstitial tissue in cases of chronic disease with cachexia. Kyrle ('10) pointed out that in diseased conditions and in underdeveloped testes, the tubules are far apart and the interstitial tissue surpasses the tubules in amount. Allen ('19) also found an increase in the quantity of interstitial tissue in rats fed upon a diet deficient in water-soluble vitamins. Allen also appears to be the only worker who found a condition of atrophy of the germ cells without hypertrophy of the interstitial tissue. This occurred in rats subjected to prolonged alcoholization.

In my own series of albino rats, there appears in no case any demonstrable increase in interstitial tissue during either acute or chronic inanition. However, in those rats which were refed after various periods of underfeeding (group VI), there is a gradual increase in the relative amount of interstitial tissue (interstitial cells) until the gland regains its normal size and structure. Thereafter, the interstitial tissue apparently decreases in amount until it reaches the normal proportion. That is, the interstitial cells appear relatively increased in number only during the regenerative period.

VI. Summary.

The results of the present investigation may be summarized as follows:

1. In the normal testis of the albino rat during the first postnatal week the (solid) seminiferous tubules consist of a single layer of parietal cells ("Sertoli cells" of v. Ebner) and a few central cells ("Ursamenzellen" of v. Ebner). After the first week, the normal postnatal development of the testis and the process of spermatogenesis proceeds essentially as described by Allen ('18).

2. In rats two days old starved for 48 to 50 hours, the testis increases markedly in weight, but mitoses are reduced in number and the normal process of histological differentiation is arrested.

3. During underfeeding for various periods beginning in rats three weeks old, mitosis continues in the cells of the seminiferous tubules; but the process of spermatogenesis is arrested at the primary spermatocyte stage, which persists even in rats over 400 days old. The spermatocytes degenerate and are resorbed, but if the number formed exceeds those destroyed the testis may increase in weight. Multinucleated giant cells are formed during the process of degeneration. The spermatogonia and Sertoli cells usually persist unaffected, except in very extreme cases, where a complete degeneration and disintegration of the seminiferous epithelium may occur. If the underfeeding begins after sexual maturity, the seminiferous tissue is much more resistant and normal spermatogenesis may persist for a long time.

4. Acute inanition in adult rats, with 30 to 47 per cent loss in body weight, produces degenerative changes in a few, irregularly scattered, tubules. All the other tubules show apparently normal structure and spermatogenesis. The degenerative changes are initiated by a desquamation of the epithelial cells into the lumen of the seminiferous tubule, followed by pycnosis and karyolysis. The process involves first the spermatids and spermatozoa, then the secondary and primary spermatocytes, and finally the spermatogonia. The Sertoli cells are the most resistant. During the degenerative process, multinucleated giant cells arise, apparently by fusion of the degenerating spermatocytes.

5. During inanition, mitosis is very persistent in the seminiferous epithelium, both in young and adult rats. It may occur even in tubules where nearly all the cells are more or less degenerated. Amitosis was not observed. A condition resembling synizesis was frequently observed in the spermatids in both controls and test rats.

6. Refeeding after prolonged inanition (beginning in rats at three weeks of age and extending to twelve to twenty weeks) results in a rapid improvement in the structure of the testis, although it may lag behind in weight for a while during the preliminary stages of reconstruction. Spermatogenesis returns to normal in a short time, and spermatozoa appeared in 37 days.

7. There is a definite hypertrophy of the interstitial tissue and an increase in the number of interstitial cells of the testis during the regenerative period on refeeding after inanition (in growing rats). No hypertrophy of the interstitial tissue was found accompanying atrophy of the seminiferous epithelium during

inanimation in either young or adult rats. The structure of the interstitial tissue is apparently normal during acute and chronic inanimation, except in extreme cases, where degenerative changes in the cells may occur.

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Table I.

Individual number, age and condition, gross body weight and weight of the testes in the albino rats used for histological study.

<u>Rat Number</u>	<u>Age, etc.</u>	<u>Final Body Weight</u>	<u>Weight of testis</u>	<u>Per cent of body weight</u>
<u>Group I -- Normal controls.</u>				
		<u>grams</u>	<u>grams</u>	<u>per cent</u>
S1 1.4	Newborn	5.1	0.0029	0.027
S1 2.5	4 days	7.1	0.0061	0.085
S1 1.2	4 days	7.0	0.0045	0.064
S1 1.3	7 days	9.1	0.0200	0.219
S1 1.5	14 days	14.7	0.0780	0.529
S1 6.0	21 days	20.6	0.0920	0.446
K 8.1	30 days	26.0	0.1154	0.443
S1 7.1	43 days	36.2	0.3940	1.088
St 5.2	56 days	77.0	---	---
S1 10.1	56 days	48.2	0.5460	1.112
S1 11.1	63 days	58.3	1.0030	1.720
S1 8.2	70 days	140.0	2.6930	1.923
S 14	450 days	314.0	2.7545	.877

Table I (Continued)

<u>Rat Number</u>	<u>Age, etc.</u>	<u>Final Body Weight</u>	<u>Weight of testis</u>	<u>Per cent of</u> <u>body weight</u> <u>per cent</u>
		<u>grams</u>	<u>grams</u>	
<u>Group II -- Acute inanition in rats starting 2 days after birth.</u>				
Si 1.1	Starved 48 hrs.	4.010 (loss 25%)	0.0043	0.107
Si 2.3	Starved 40 hrs.	4.215 (loss 21%)	0.0051	0.121
Si 2.6	Starved 50 hrs.	4.100 (loss 25%)	0.0045	0.109
<u>Group III -- Held at constant body weight by underfeeding from three weeks of age.</u>				
St 9.36	Maint. 21-51 days	30.5	0.0958	0.31
St 12.71	Maint. 21-67 days	31.2	0.1056	0.50
S 7.31	Maint. 21-70 days	31.4	0.3310	1.05
S 7.32	Maint. 21-71 days	28.8	0.2100	0.73
S 11.65	Maint. 21-73 days	22.5	(R) 0.1860	0.83
			(L) 0.0496	0.22
<u>Group IV -- Retarded by underfeeding for long periods beginning at three weeks of age.</u>				
St 44.1	Underfed 21-223 days	63.9	1.097	1.72
St 44.7	Underfed 21-418 days	40.9	1.187	2.90
St 44.4	Underfed 21-428 days	47.0	1.2968	2.75
M 29.109	Underfed 70-314 days	68.7	1.099	1.58

Table I (Continued)

<u>Rat Number</u>	<u>Age, etc.</u>	<u>Final Body Weight</u>	<u>Weight of testis</u>	<u>Per cent of</u>
		<u>grams</u>	<u>grams</u>	<u>body weight</u>
				<u>per cent</u>
<u>Group V -- Adult acute inanition.</u>				
S 25	Starved 9 days	198.3 (loss 35%)	1.479	0.75
S 26	Starved 8 days	171.5 (loss 39%)	1.905	1.11
S 27	Starved 8 days	219.0 (loss 30%)	2.430	1.11
S1 9.1	Starved 11 days	114.0 (loss 47%)	1.468	1.28
<u>Group VI -- Refed after underfeeding from 3 weeks to age indicated.</u>				
St 12.53	85 days Refed 3-1/2 days	44.9	0.2010	0.45
St 10.26	96 days Refed 14 days	86.3	0.2672	0.31
S 12.52	109 days Refed 37 days	117.4	1.1848	1.01
S 33.118	346 days Refed 206 days	219.0	2.5182	1.14

IX. Figures and explanation.

The drawings were made with the aid of a camera lucida. The unit of magnification is 550 diameters for all the figures showing the normal histogenesis of the testis. Figures 9 and 11 are drawn at a magnification of 500 diameters; figures 10 and 12 at a magnification of 240 diameters. All the material stained with Heidenhain's iron-alum haematoxylin had been fixed in Carnoy's fluid (No. 1). The remaining material had been fixed in either Bouin's or Zenker's fixatives.

Figures 1 to 7 show normal development in the epithelial wall and the growth of the tubules in albino rats from birth to 8 weeks of age. All X 550.

Figures 8 to 13 show changes in the seminiferous epithelium and size of the tubules brought about by experimental conditions.

Fig. 1. Transverse section of tubules from the testis of a normal newborn rat (Si 1.4). Section 6 micra thick; stained with iron-alum-haematoxylin and acid fuchsin. X 550. Note the presence of a single layer of parietal cells, P.C., and the large central cells, C.C., in the center of the tubule. The large, deeply-staining cells in the mesenchymal tissue on the right are apparently the precursors of the interstitial cells, I.C. Blood vessel, B.V., Mesenchymal cell, M.C. Each tubule is surrounded by a membrana propria, M.P.

Fig. 2. Transverse sections of two tubules from the normal testis at 4 days (Si 2.5). Section 6 micra thick; stained with iron-alum-haematoxylin. X 550. The number of parietal cells, P.C., has increased, while the central cells, C.C., are less frequent. Membrana propria, M.P. The interstitial cells, I.C.,

are beginning to group themselves together in certain places.
 Blood vessel, B.V. Mesenchyme cell, M.C.

Fig. 3. Transverse section of two tubules from the normal testis at 7 days (Si 1.3). Section 6 micra thick; stained with iron-alum-haematoxylin. X 550. Mitotic activity is apparent. A spermatogonium in metaphase is well shown, Sp.C. Germinative cells are all alike (Allen's type A). Membrana propria, M.P. Mesenchyme cells, M.C. Interstitial cells, I.C. Note the interstitial cell, M.F., showing karyokinesis.

Fig. 4. Section of a tubule from the normal testis at 14 days (Si 1.5). Section 6 micra thick; stained with iron-alum-haematoxylin and acid fuchsin. X 550. Cells of (Allen's) types A and B are abundant and a few cells of type C are present, A., B., C. Sertoli cells, S.C., are beginning to assume their adult position and structure. Membrana propria, M.P.

Fig. 5. Sections of a tubule from a normal testis of 21 days (Si 6.0). Section 10 micra thick; stained with iron-alum-haematoxylin. X 550. All types of cells, A., B. and C., are present. Primary spermatocytes, P.S., have appeared. Sertoli cells, S.C. Membrana propria, M.P. Note the formation of a lumen, L., in the tubule.

Fig. 6. Section of a tubule from a normal testis at 4 weeks (K 8.1). Section 10 micra thick; stained with iron-alum-haematoxylin. X 550. Presents the same picture as the preceding figure, but also shows a marked increase in the number of primary spermatocytes, P.S.

Fig. 7. Portion of a transverse section of a tubule from a normal testis at 8 weeks (St 5.2). Section 10 micra thick; stained with iron-haematoxylin. Note the gradual transition from the spermatogonia to the mature spermatozoa. This corresponds very closely to the condition found in the adult testis. Sertoli cell, S.C. Spermatogonia, S. Primary spermatocytes, P.S. Secondary spermatocytes, S.S. Spermatids, Spt. Spermatozoa, Sp. Membrana propria, M.P.

Fig. 8. Section of three tubules from the testis of a rat 4 days old (Si 2.6) which had been subjected to acute inanition for 50 hours. Section 6 micra thick; stained with iron-alum-haematoxylin and acid fuchsin. X 550. Note that the structure resembles that of the newborn more closely than that normal at 4 days. Compare with figures 1 and 2. Central cells, C.C. Parietal cells, P.C. Membrana propria, M.P. Interstitial cells, I.C. Mesenchyme cells, M.C.

Fig. 9. Section of a tubule from a rat (S 12.71) held at constant body weight by underfeeding from three to ten weeks of age. Section 10 micra thick; stained with haematoxylin and eosin. X 500. Degenerating primary spermatocytes, D.P.S., showing the homogeneous, deeply acidiphilic staining reaction of the cytoplasm. Note the persistence of mitotic figures, M.F.; the normal Sertoli cells, S.C., and spermatogonia, S. A multinucleated cell, Mn.C., typical of those appearing as a result of chronic inanition. Degenerating cytoplasm, D.C. Membrana propria, M.P.

Fig. 10. Section of a portion of the atrophic testis from a rat (S 11.65) subjected to chronic inanition from three to ten weeks of age. Section 10 micra thick; stained with haematoxylin and eosin. X 240. The central tubule shows the extreme stage of degeneration (described in text), a complete nearly homogeneous mass of necrotic tissue. The surrounding tubules show various stages of degeneration. Note the mitotic figures, M.F., in the tubule to the right and the apparently normal Sertoli cells, S.C. A slight hyperplasia of the interstitial cells, I.C., appears. Blood vessel, B.V.

Fig. 11. Section of a tubule from the testis of a rat (St 44.4) stunted by underfeeding from three weeks to 428 days of age. Section 10 micra thick; stained with haematoxylin and eosin. X 500. Note the degenerating primary spermatocytes, D.P.S.; the multinucleated cell, Mu.C., in the lumen. Sertoli cells, S.C., and spermatogonia, S., are apparently normal. Membrana propria, M.P. Comparison with figure 9 shows a decrease in the size of the tubule.

Fig. 12. Oblique section of a testis from an adult rat (S 25) subjected to acute inanition for 9 days. Section 10 micra thick; stained with haematoxylin and eosin. X 240. Multinucleated giant cells, M.G.C., occupy the center of the tubule. Débris composed of degenerating cells of various kinds fills the tubule. Spermatozoa, Sp. Spermatids, Spt. Spermatogonia, S. Membrana propria, M.P. The Sertoli cells, S.C., still occupy their normal position, but show atrophic and degenerative changes.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 13. Portion of transverse section of the testis from a rat (S 12.52) underfed from three to ten weeks of age and then refed for 37 days. Section 7 micra thick; stained with haematoxylin and eosin. X 550. Note the large number of normal primary spermatocytes, P.S., and spermatozoa, Sp. Spermatogonia, S., showing mitotic figures are abundant. Sertoli cell, S.C. Membrana propria, M.P.

Fig. 14. A to K represent various stages in the interstitial (mesenchymal) cells of the testis during normal development, inanition and refeeding. All magnified X 550. A, normal newborn (Si 1.4). B, normal at 4 days (Si 2.5). C, normal at 7 days (Si 1.3). D, normal at 21 days (Si 6.0). E, normal at 4 weeks (K 8.1). F, normal at 8 weeks (St 5.2). G, normal adult (S 14). H, underfeeding, 3 to 10 weeks of age (S 12.71). I, extreme atrophy in underfeeding from 3 to 10 weeks of age (S 11.65). J, underfeeding from 3 weeks to 428 days of age (St 44.4). K, refed 37 days after underfeeding from 3 to 10 weeks of age (S 12.52).

Fig. 14