A STUDY OF THE FUNCTION OF THE EPIDIDYMIS

IV. THE FATE OF NON-EJACULATED SPERMATOZOA IN THE GENITAL TRACT OF THE MALE GUINEA-PIG

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(With One Text-figure.)

INTRODUCTION.

The results of recent investigations (Young, 1929 a, b, 1931) on the nature of changes undergone by spermatozoa during their passage through the epididymis in the guinea-pig have demonstrated that immature cells are constantly being carried into this organ from the seminiferous tubules, and that much of the time consumed in passing through the 9–9½ ft. long ductus epididymidis is necessary for a completion of their development. After full maturity has been attained, however, there is no influence which preserves their vitality indefinitely, and they age and become incapable of functioning. Such being the case, it seemed, especially in males which are not constantly eliminating spermatozoa in copulations, that there must be some provision for the removal of non-ejaculated spermatozoa which insures the presence of a supply of viable spermatozoa at the distal end of the vas deferens (Young, 1929 b).

The paper cited above was not the first to contain the suggestion that there is a constant loss of spermatozoa from the male genital tract over and above those discharged during copulations. Numerous other writers have postulated such an elimination as a logical sequence to the continual production of spermatozoa which is known to occur in the testes of many mammals.

Benoit (1926, for the guinea-pig), Baldwin (1928, for man), Nakano (1928, for the bat), and Oslund (1928, for the guinea-pig, dog, rat, and man) either assume or state definitely that non-ejaculated spermatozoa are eliminated in the urine.

Exner (1904) and Königstein (1908) have suggested for man that spermatozoa enter the seminal vesicles where their degeneration and resorption occur. Any destruction of spermatozoa in the seminal vesicles of the guinea-pig would seem improbable, however, if it is true, as several investigators have claimed, that spermatozoa are not normally found in the seminal vesicles of this species (Minot, 1884; Wertheimer and Dubois, 1922; Fisher, 1923; Warnock, 1923; Armitstead, 1925).

According to a statement in Marshall’s (1922) Physiology of Reproduction,
The spermatozoa which are not ejaculated degenerate. The tails break off and undergo a gradual liquefaction. The end products are ultimately absorbed by the epithelial cells of the seminal vesicles, and perhaps by the cells of the vasa deferentia or of the testis itself." Priesel (1924) expresses a somewhat similar opinion in suggesting that non-ejaculated spermatozoa, particularly those which are abnormal, are liquefied and resorbed within the head of the epididymis.

In addition to his suggestion that spermatozoa are eliminated in the urine, Nakano has also expressed the opinion that, in the bat, some spermatozoa penetrate the epithelial lining of the epididymis, and become lodged in the underlying connective tissue where their degeneration occurs.

The last theory of the manner in which non-ejaculated spermatozoa are eliminated from the male genital tract would seem to have been satisfactorily refuted, but is mentioned because of the recent attention it has received. Regaud and Tournade (1911) described large cellular elements in the lumen of the epididymis of a rat, which they regarded as desquamated epithelial cells containing spermatozoa and spermatozoan remains in the process of being resorbed. Guieysse-Pellissier (1911), using the guinea-pig, examined the efferent passages which had been subjected to operative interference, and found what he called a phagocytosis of spermatozoa by cells loosened from the epididymal epithelium. The term "spermiophagie," used to designate the destruction of spermatozoa in this manner, seems to have been employed first by Wegelin (1921), and to have been adopted subsequently by Morgenstern (1923, 1924), Lehner (1924 a, b), Priesel (1924) and Nemiloff (1926). Wegelin and Morgenstern considered the large mono-nucleated and multi-nucleated giant cells, found principally in the lumina of the efferent ducts, to be macrophage elements derived from the epididymal epithelium. Lehner and Nemiloff agree with Wegelin in calling these elements "spermiophages," but Lehner believes that they are derivatives of the Sertoli cells, while Nemiloff suggests that they may take their origin from the basal cells in the epididymal epithelium and from resting wandering cells in the underlying connective tissue.

Lehner, Morgenstern, Nemiloff, Priesel and Wegelin thought this was the method, possibly common under pathological conditions, whereby imperfect spermatozoa are destroyed. Lehner, Morgenstern and Nemiloff also consider that the phenomenon occurs normally for imperfect spermatozoa, and the two latter, especially Morgenstern, feel that at least some of the non-ejaculated normal spermatozoa may be destroyed in this manner.

Credit for what would appear to be a satisfactory disposition of the question belongs to Akiyoshi (1924), Sternberg (1924) and Stieve (1925). All have pointed out (1) that much of the material in which "spermiophages" were seen had been removed from pathological or senile animals, and (2) that the so-called "spermiophages" are nothing but degenerating germinal elements which had been sloughed off the germinal epithelium under abnormal conditions and carried into the lumen of the epididymis. Confirmation would seem to come from observations reported by Young (1927) in his study of the degenerative changes in the germinal epithelium of the guinea-pig testis following heat injury.
It will be realised from this variety of theories with respect to the manner in which non-ejaculated spermatozoa are eliminated from the genital tract, that more evidence was needed before any one could be depended upon for the completion of the account of the post-testicular history of spermatozoa in the guinea-pig which was desired. A new series of experiments was therefore undertaken.

The result, as announced in a preliminary communication (Young and Simeone, 1930), was the rejection of the older hypotheses to the extent that they apply to the guinea-pig, with the exception of certain features of the opinions expressed by Marshall and Priesel. In their places the suggestion was made that a liquefaction or dissolution of non-ejaculated spermatozoa is constantly taking place, particularly within the vas deferens. A description of the experiments which have led to this conclusion are contained in the following section.

EXPERIMENTAL.

(a) Examination of the urine and the urethra.

The first experiments were planned with the purpose of re-investigating the possibility, proposed particularly by Oslund (1928), that spermatozoa are continually being forced into the urethra and then voided with the urine. A variety of procedures were employed.

Urine collected directly from the bladder was examined. Samples of urine were obtained by applying external pressure in the region of the urinary bladder while the male was under a light ether anaesthesia. Occasionally no urine was obtained, but the method was successful in the majority of cases. The urine was collected in 25 c.c. beakers, diluted with an equal volume of salt solution, and examined microscopically. The first few samples were subjected to centrifugation, but the large quantity of sediment made such a procedure impractical.

Twenty-one samples collected at 24-hour intervals were obtained from four males. No normal or degenerate spermatozoa were found in any one of the samples.

It then seemed desirable to examine the urine from males which had recently mated. Five males were allowed to mate with females during the oestrus. Samples of urine were then obtained immediately in the manner described above. The urine from one of the five males contained countless spermatozoa. The urine from the others contained between two and ten spermatozoa per smear. Urine collected from these males 24 hours after the copulations contained no spermatozoa. It would seem, therefore, that while spermatozoa can be found in the urine immediately after a copulation, the period during which they are voided is short.

It was then felt that males which are not allowed to mate may experience periodic discharges of spermatozoa into the urethra. If this is true, such spermatozoa may well have been missed in the twenty-one random samples referred to above. Provision was made, therefore, to collect and examine all the urine passed over a period of several weeks.

False bottom cages made of fine-meshed galvanised wire screening were constructed in the form of a cylinder fitting snugly over a 10 in. glass funnel, the stem
of which was cut short. Each cage was mounted on an ordinary tripod, the legs of which were bent so that the end of the funnel actually touched a piece of screening which covered a small stender dish. The screening prevented oats and other debris from dropping into the urine. For the attachment of the feeding pans, openings were cut on opposite sides of the cages, the cut being folded over and fastened to the outside to prevent the possibility of injury to the animal. The cages were cleaned thoroughly at 48-hour intervals.

The practicality of such a cage was tested in the following manner. An animal was confined in a cage for 3 days. During this time a considerable quantity of hair and faecal material accumulated on the screening and in the funnel. 2 c.c. of a very dilute spermatozoon suspension were then poured into the cage. Several drops reached the collecting dish, which contained 10 c.c. of urine. This suspension was then examined for the presence of spermatozoa. Numbers of these cells were detected with ease in each field examined.

Five normal males were then confined in these cages for periods of from 10 to 46 days' duration. Their urine was examined microscopically as soon as possible after its elimination, at least once a day for three animals and twice a day in the case of two. Three smears were made from each collection, the medicine dropper being washed after each smear. The collecting dish was washed and replaced immediately after each examination and no urine was lost.

No spermatozoon was ever found in the urine collected from two animals throughout periods of 10 and 32 days respectively. In a third animal whose urine was collected throughout a period of 46 days, no spermatozoon was found during the first 34 days. On the 35th day and again on the 41st day, an occasional spermatozoon was found. In a fourth animal whose urine was collected throughout a period of 33 days, a few spermatozoa were found on the 9th, 12th, and 22nd days. In the fifth male, whose urine was collected throughout a period of 33 days, a few spermatozoa were found on the 22nd day. In no sample in which spermatozoa were found were they more numerous than one to a smear; and, in most samples in which they were present, they were not found except in one out of every three to five smears. It seemed, therefore, that any elimination of spermatozoa in the urine is the exception rather than the rule, and that the number which are voided in this manner is entirely too small to account for the extent of spermatozoon elimination which must be occurring.

The possibility remained, however, that there had been no loss of spermatozoa from these animals during the experimental period. It seemed desirable, therefore, to examine the urine from males throughout a period during which it was known that spermatozoa were disappearing from the genital tract. This was done in the case of two males. No more were used, because the data obtained from these confirmed previously obtained results so completely.

The heads of the epididymides were ligatured in such a way that spermatozoa could no longer pass beyond the vasa efferentia. At the time of the operation the epididymides were well filled with spermatozoa. The males were then placed in false-bottom cages. Their urine was collected and examined twice daily over a
period of 30 days. At the end of this period the males were killed and their epididymides examined as before.

In the case of one male not a single spermatozoon was found in the urine voided during the 30 days of the experiment; and yet the epididymides were more than half emptied at the end of this time. In the case of the second male, a few spermatozoa were found on each of the 3rd and 4th days after the operation, but at no other time during the remaining 26 days of the experiment. Further proof was obtained, therefore, for the conclusion suggested above that normally there is no general elimination of spermatozoa in the urine.

The experiment is of interest for the information it gives with respect to the nature of the force which is responsible for the distally directed current of spermatozoa through the epididymis. According to Oslund (1928), the continuous production of secretion within the seminiferous tubules is sufficient to maintain the current in which spermatozoa are carried toward the vas deferens. If this were the only force involved, it would be difficult to account for the partial emptying of the epididymides which occurred in those animals in which there was no connection between the seminiferous tubules and the epididymis. The suggestion seems more probable that normally this distally directed current is maintained in part by the continuous elaboration of secretion within the seminiferous tubules, in part by the action of the cilia present in the epithelium of the vasa efferentia, and in part by some neuro-muscular action.

A last factor which the above described experiments had not taken into account was the remote possibility that spermatozoa may be forced into the urethra, where their destruction occurs before they can be eliminated in the urine. This possibility was disposed of by the results from two series of observations.

In the first, the urethrae from four males were examined for the presence of spermatozoa. The distal end of the urogenital tract, beginning with the distal end of the vas deferens, was divided into five parts, each less than 1 cm. in length. Each part was then macerated in 10 c.c. of physiological salt solution, and drops of the suspension were examined microscopically. Without exception, many spermatozoa were found in the suspension from the distal end of the vas deferens. In the urethra, on the other hand, they were found in small numbers in the suspensions from the two anterior sections, and only rarely in suspensions from the more posterior sections. The presence of spermatozoa in the anterior part of the urethra may be attributable to the impossibility of effecting a separation of the vas deferens and urethra without forcing some of the contents of the former into the latter, rather than to the escape of spermatozoa from the vas deferens prior to the death of the animal.

In the second series of experiments, we investigated the possibility that the urine or some substance from one of the accessory sex glands (seminal vesicle, prostate, and Cowper's gland) might cause a destruction of spermatozoa forced into the urethra from the vas deferens.

The genital tract was removed from the male. The spermatozoon suspension obtained by macerating each epididymis in 20 c.c. of Locke's solution was used
as a standard suspension. Portions of the accessory sex glands were then macerated, each in 5 c.c. of Locke's solution, following which the larger fragments of tissue were removed from the suspensions. The anterior lobes of the prostate gland were separated from the middle and posterior lobes because of the histological and physiological differences known to exist between them (Walker, 1900, 1910; Engle, 1926). A solution of urine was prepared by adding a volume of urine obtained directly from the bladder to an equal volume of Locke's solution. A sufficient quantity of spermatozoan suspension was then added to each of the solutions described above to make a series of suspensions in which the concentration of spermatozoa was approximately equal. A control was prepared by adding an equivalent quantity of spermatozoan suspension to 5 c.c. of Locke's solution. Each suspension was examined immediately for the concentration and condition of spermatozoa. The dishes were then covered and allowed to stand at room temperature. They were examined after 24 hours and again after 48 hours, following which they were discarded, except that the suspension of spermatozoa in urine was examined for 4 days.

Although the spermatozoa in Locke's solution remained motile for 24 to 48 hours longer than those contained in any other solution, the latter had no destructive effect such as a dissolution which could be thought of as causing the complete disappearance of spermatozoa within the genital tract. In the solutions prepared from the macerated seminal vesicles, prostate gland, and Cowper's gland, spermatozoa were readily identifiable after 48 hours; and in the solution of urine they were recognizable after 4 days. The early loss of motility on the part of spermatozoa contained in the solutions prepared from the accessory sex glands is probably a consequence of putrefactive changes rather than of any specific action by substances from these glands.

These results are what were expected on the basis of the rôle which the secretions from these glands have in the reproductive process. It is concluded, therefore, that if spermatozoa were forced into the urethra from the vas deferens, no substance in this part of the urogenital tract would cause their immediate destruction, and they would be voided with the urine, which they are not.

From the standpoint of the problem as a whole, the results described above confirm the idea advanced previously that, in the guinea-pig, there is a constant distally directed current of spermatozoa through the epididymis and vas deferens. The same observations have provided supporting evidence for the suggestion that the removal of spermatozoa from the distal end of the tract is not dependent upon ejaculations, but that in some other manner spermatozoa are being removed continually, even in sexually inactive animals. Lastly, unequivocal evidence has been provided that spermatozoa do not get into the urethra to be voided with the urine. At the same time, no clue as to the means by which spermatozoa are eliminated was obtained, and since the numerous modifications of the method used seemed to have exhausted the possibilities as far as this approach was concerned, it was thought desirable to see what could be learned from a microscopic study of the tissues involved.
(b) Histological study.

The portion of the urogenital tract where the vasa deferentia, the seminal vesicles, the prostate gland, and the urethra converge, as well as portions of the vasa deferentia and the cauda epididymidis, were removed from five normal males which had not mated for at least a week, and sectioned serially. The tissues were fixed in either Carnoy’s fluid (A), Bouin’s fluid, or Allen’s modification of Bouin’s fluid; cleared in either synthetic oil of wintergreen or oil of cloves; imbedded in paraffin; sectioned from 7 to 12 micra in thickness; and stained with Harris’ hematoxylin and eosin.

Fig. 1. Photomicrograph (low power magnification) of distal section of vas deferens showing portions of twelve masses of bunched degenerating spermatozoa.

Examination of the tissues not only revealed much that was of interest for the problem, but it also disclosed what is believed to be the fate of non-ejaculated spermatozoa.

Our attention was directed to conspicuous masses of cells contained in the lumina of the vasa deferentia (Fig. 1). These masses were found to be largest and most numerous in the distal ends of the vasa deferentia, just anterior to the point where they are joined by the seminal vesicles to form the ejaculatory ducts. In this region as many as ten to fifteen masses can frequently be seen in a single cross-section. As more proximally located sections are studied, the masses can be observed
to become smaller in size and less numerous; until, finally, in the most distal coils of the cauda epididymidis, they disappear altogether.

The masses are composed of such a dense, entangled mass of cells, that careful study of them is difficult in fixed preparations. Each can be seen, however, to be composed of disintegrating spermatozoa and other debris which is strongly eosinophilic. The latter is thought to be a product of cytolytic changes which the spermatozoa are undergoing. The masses contain very few, if any, sloughed off epithelial elements, groups of which are easily distinguishable; they are composed essentially of spermatozoa, and spermatozoon remains apparently undergoing a process of liquefaction or dissolution.

In order to learn more concerning the composition of these masses and to determine definitely that they are of regular occurrence, the contents from various levels of the vasa deferentia and the epididymides from four normal males were examined in vitro. The earlier observations received immediate confirmation. The masses were found to be spindle-shaped to spherical, and to be composed entirely of non-motile, degenerate spermatozoa and their remains. When the masses are washed and teased apart, the spermatozoa can be seen to be in all stages of degeneration, many being without heads. The centres of the masses contain much debris, the origin of which is uncertain, but which is believed to be the degenerating spermatozoa.

Not all the non-motile spermatozoa were found in these masses. A large number of isolated, dead spermatozoa were found in suspensions from both the vas deferens and the epididymis.

The location of the masses in the distal end of the genital tract, the frequency of their occurrence in the vas deferens compared with their absence or rarity in the epididymis, and their composition of numerous, non-motile, degenerating spermatozoa, suggests strongly that they are the visible expression of the process by which non-discharged spermatozoa are removed from the vas deferens in the guinea-pig.

The suggestion that a liquefaction or dissolution of spermatozoa in the vas deferens is the normal manner in which they are eliminated has been found in only one place in the literature reviewed. As was noted in the introduction, Marshall (1922) suggests that spermatozoa which are not ejaculated undergo a gradual liquefaction. On the whole, he feels that the end products are absorbed in the seminal vesicles, although he suggests as a possibility that they may likewise be absorbed by the epithelial cells of the vasa deferentia. If Marshall means that the process of liquefaction as well as absorption occurs in the seminal vesicles and vasa deferentia, we differ from him as far as the guinea-pig is concerned (1) in feeling that the liquefaction is confined to the vasa deferentia and epididymides, and (2) in being undecided as to whether the end products of liquefaction are absorbed or removed in some other manner.

It is thought that at least two factors may be responsible for the wholesale destruction of spermatozoa which has been observed. Previous studies (Young, 1929a, b, 1931) have shown that spermatozoa enter the epididymis as immature
cells and attain their full maturity during the passage through this organ. But after these ripening changes have occurred, there is no influence which preserves them indefinitely, and they age and become incapable of functioning. The death and degeneration which have been observed in this study are believed, therefore, to be a consequence of age and a natural sequence in a chain of events which is interrupted only if the spermatozoa are discharged. The apparent increase in the number of dead and degenerate spermatozoa found in the distal end of the vas deferens provides some basis for this suggestion.

Another factor which must be considered as of equivalent, if not greater, significance than that of spermatozoon age in the degeneration which has been seen to occur, is the higher temperature which probably prevails at the distal ends of the vasa deferentia where these structures are contained in the abdominal cavity rather than in the scrotal sacs (Moore and Quick, 1923; Heller, 1929). That this factor is to be regarded as one which may be superimposed upon that of spermatozoon age, rather than as one which acts alone, is suggested by the observation that many isolated degenerate spermatozoa and a few masses are found in the extreme distal end of the epididymis which is located well down in the scrotal sac. A more careful investigation of this point should be made.

In addition to detecting the numerous masses of degenerating spermatozoa to which so much importance has been attached, other observations were made during the study of sections of the genital tract which are of interest for the problem.

The small calibre of the constricted distal end of the vas deferens was conspicuous. The minuteness of this opening may prevent the flow of material from the vas deferens into the urethra except at the time of ejaculations. The condition may account for the practical absence of spermatozoa from the urethra and the urine.

No evidence for a penetration of the epithelium by spermatozoa such as has been described by Lehner (1924a, b) for the guinea-pig, Nemiloff (1926) for the starved cat, and Nakano (1928) for the hibernating bat, could be found in any part of the genital tract from the caput epididymidis to the urethra. In some sections spermatozoa were found in the areolar connective tissue which lies between the various structures in this part of the urogenital system. These, however, must have been carried across the surface of the section at the time of sectioning or staining, because characteristic "rouleaux" of spermatozoa, such as are found normally in the epididymis, can be seen among other spermatozoa; and they could not have penetrated the epithelium. It is not believed, therefore, that this can be a method of spermatozoon elimination in the guinea-pig such as Nakano has suggested for the bat.

Examination of the seminal vesicles in living animals and in fixed preparations did not reveal any spermatozoa in these organs. It is probable, therefore, that no degeneration of spermatozoa occurs in the seminal vesicles such as Exner (1904) and Königstein (1908) have described for man.

Lastly, a careful examination was made for the presence of phagocytes which might account for the disappearance of spermatozoa, but no more than an occasional cell of this type was found. It is concluded that any phagocytosis which occurs is of no significance for the removal of spermatozoa from the genital tract.
DISCUSSION.

The chief significance of the results obtained from the experiments described in the preceding section is that they have yielded information which, when added to that obtained previously (Young, 1929 a, b, 1931), enables us to give what is at least a bare outline of all the changes undergone by spermatozoa during their passage through the epididymis, and of the importance of this organ for the spermatozoa themselves.

It now seems, as far as the guinea-pig is concerned, that spermatozoa are carried from the seminiferous tubules into the ductus epididymidis as immature cells. They are then carried distally in a current which may be caused in part by the constant production of seminal fluid in the testis, in part by ciliary action within the vasa efferentia, and in part by the action of the smooth musculature present in the distal part of the ductus epididymidis and the vas deferens. During this time the experiments seem to show that they are contained in an environment in which stimuli to their development similar to those in the testis are present, and that changes which are responsible for the attainment of an optimal condition for effecting fertilisation continue within the substance of their protoplasm. If the spermatozoa which have attained their full maturity are not discharged in copulation, there is no influence which preserves them indefinitely, and regressive changes begin which end finally in their liquefaction or dissolution in situ. As would be expected, this liquefaction is most apparent among those spermatozoa which have reached the distal end of the vas deferens.

While the above is an account of the general course of spermatozoon history after these cells have left the testis, there are still many questions which must be disposed of before the subject can be regarded as having been investigated exhaustively.

Certain of these which relate to the progress of spermatozoa through the epididymis have already been mentioned (Young, 1931) as being under investigation, viz. the factors which influence the rate of progress of spermatozoa through the epididymis, the relation between rate of progress through the epididymis to the physiological condition of spermatozoa present at the distal end of the vas deferens, and the reproductive capacity of over-ripe spermatozoa.

A question which has not been raised heretofore in connection with this investigation has to do with the fate of the products of spermatozoon liquefaction. Do these products pass into the urethra to be voided with the urine, or are they resorbed through the epithelial lining of the vas deferens and epididymis? The first possibility has not, as far as can be learned, received the attention of any investigator. Parenthetically, it would seem that a loss of fluid from the vasa deferentia into the urethra would be accompanied by more of a loss of spermatozoa in the urine than was detected in the experiments reported above. The second possibility has received attention to the extent that the resorptive ability of the epididymal epithelium has been studied. With the exception of Priesel (1924), however, those who have worked in this field have not suggested any relationship between a resorptive activity
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...and the manner in which non-ejaculated spermatozoa are removed from the genital tract.

Von Möllendorff (1920, 1924) and Wagenseil (1928) have expressed the opinion that the cells lining the lumen of the vasa efferentia are capable of resorbing liquid substances present in these tubules. Kyrlé and Schopper (1915), Romeis (1922), Stieve (1922), Priesel (1924), Redenz (1924), and Nassonov (1927) believe that the epithelial cells in the head of the epididymis may also function in this manner. Belfield and Rolnick (1927), on the other hand, agree that a resorption of fluid substances occurs, but suggest that such a resorption occurs in the body and tail of the epididymis rather than in the head. An evaluation of these opinions as to the region where resorption occurs is further complicated by von Lanz' (1926 a, b) denial of a resorptive activity in any part of the organ. In view of this situation and the failure of anyone to have given particular attention to the vas deferens where spermatozoon liquefaction has been suggested as being the most general, it would seem that the present is not the time to add the idea of a resorption of the products of spermatozoon liquefaction to the theory of the post-testicular history of spermatozoa which has been advanced.

The many differences between the theory of the epididymal spermatozoon relationship advanced in this series of papers, and the theories advanced by Redenz, von Lanz, and Belonoschkin (see preceding paper for bibliography) have already been emphasised (Young, 1931) and need not be reviewed here. The suggestion we have made to the effect that the epididymis is an organ in which a certain predestined and necessary course of spermatozoon development is free to continue, rather than as an organ whose secretions exert certain specific ripening and preserving influences on spermatozoa as these cells pass through it, is strengthened by our ability to observe the full extent of the regressive changes which had been postulated to occur.

How far the observations recorded above will apply to other species is not known. We have wondered, however, if the numerous dead spermatozoa found by Amantea and Krzyszowsky (1921) and Krzyszowsky and Pawlow (1927) in the semen of the dog, and by Walton (1930) among spermatozoa removed from the vas deferens of the rabbit, may not be expressions in these animals of the conditions we have observed in the guinea-pig. Until other species have been studied, however, the suggestions made in this and the preceding papers must not be considered as applying beyond the guinea-pig.

CONCLUSIONS.

1. The prevailing theories that non-ejaculated spermatozoa are voided with the urine, or that they penetrate the epithelial lining of the epididymis to be resorbed in the underlying connective tissue, or that they degenerate and are resorbed in the seminal vesicles, have received no support from a variety of observations on the guinea-pig.
2. Instead, spermatozoa which have attained their full functional maturity and are not discharged in copulations, undergo regressive changes which end in their death and subsequent liquefaction within the epididymis, and particularly within the vas deferens. As would be expected, the number of dead and degenerating spermatozoa appears to be greatest among those removed from the distal end of the vas deferens. The cause of the liquefaction is suggested as being some change associated with the ageing process, or possibly the latter aided by some action of the higher temperature, which is probably encountered in that portion of the vas deferens located within the abdominal cavity.

3. On the basis of this and earlier studies of the changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea-pig, it is concluded (1) that spermatozoa are carried out of the testis into the ductus epididymis while they are functionally immature, (2) that much of the time consumed in passing through this 9–9½ ft. long tubule is necessary for a completion of their development, and (3) that the development which occurs is the result of changes within the substance of the spermatozoa which start while these cells are still a part of the germinal epithelium, and (4) that once full spermatozoon is attained in a male which is not copulating frequently, there is no influence which preserves them in an optimal functional condition; they age and ultimately disappear by a process of liquefaction or dissolution in situ.

REFERENCES.

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