Numerical Relation of Spermatoza to Sertoli Cells

by Florence S. Hague

1914

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Approved Kenneth M. Allen.

I take pleasure in acknowledging the suggestions and assistance of Professor W. J. Baumgartner and Dr. B. M. Allen under whose direction this work was done.

Numerical Relation of Spermatozoa to Sertoli Cells.

This study was started with the purpose of ascertaining the number of spermatozoa associated with each Sertoli cell in the human testis. However, on account of being unable to get material which was in the right condition, the problem was changed to lower mammals.

The tissues used were from testes of man, the cat and the rat. Nothing was known as to the age or condition,- healthy or otherwise, of the human material. The pieces which I had for sectioning had been preserved in formalin. There were also several celloidin mounts which I studied. Flemming's tri-color, Davis' cytology stain, and iron haematoxylin with acid fuchsin, and eosin as counterstains gave the best results. Delafield's haematoxylin with orgnge G, congoroth, and bordeaux red resulted in very lightly stained sections. For fixing the rat testes Mueller's, Gilson's, Bouin's, Tellyesniczky's and Zenker's fluids were used, the last two of which were the most satisfactory. The cat testes were preserved in Tellyesniczky's and Flemming's solutions. For sections of

both the rat and cat testes tricolor, iron haematoxylin with or without eosin, dilute Delafield's haematoxylin and haemlum were used. The sections varied in thickness from 6 to 40 micra.

3 The Sertoli cells are the large irregularly shaped cells in the tubules of the mammalian testes. Corresponding cells are found in lower vertebrates. There are two distinct theories as to the origin of these cells. The one, that the Sertoli cells and spermatogonia come from entirely different elements, or are two distinct cell stocks, is held by Watase, Bardeleben, Benda, Waldeyer, Walker and Embleton. The other theory is that they come from one and the same group or stock of cells. This theory has two interpretations. Stephan and Regaud believe that the Sertoli cells in some way give rise to germ cells as well as nourish them. Prenant, Schoenfeld, Bugnion, Montgomery, and Winiwarther believe that the Sertoli cells are differentiated from the germ cells near the time of the last spermatogonial divisions.

4 It is generally agreed that the spermatogonia develop by repeated and indefinite divisions from cells known as the primordial germ cells. In the cells of one of the last generations of the spermatogonia, Montgomery

and Winiwarther have observed and described a rod or crystal which passes undivided in each of two successive divisions to a daughter cell. They agree that from the cell provided with a rod a Sertoli cell develops; but Montgomery claims that the other three of the four cells resulting from these two divisions divide again, while Winiwarther says that these three themselves become primary spermatocytes by growth. The former thus gets the ratio of 24 spermatozoa to one Sertoli cell, and the latter the ratio of 12 spermatozoa to each Sertoli cell.

5 After comparatively little study of mature Sertoli cells and spermatozoa I reached the conclusion that there could not be as many as 24 spermatozoa to each Sertoli cell. I then worked on the theory that there were 12 spermatozoa to each Sertoli cell, but this too had to be abandoned, since the counts showed smaller numbers.

6 In the human material which I have the spermatozoa have nearly all been expelled. Accordingly, distinct Sertoli cells filled with spermatozoa are hard to find. I found three cells in which there were twelve spermatozoa, a few with ten or eleven, and more with eight or nine spermatozoa. However, I have no way of telling whether or

not the Sertoli cells are complete, for the sections are only 10 micra thick with the exception of the celloidin ones. The few spermatids are not grouped together, thus making counts impossible.

The rat testes were taken from two animals and were in a condition of very active production of spermatozoa. In the rat the mature Sertoli cells are long and slender, and have the appearance of striations. (Fig I & II) The spermatocytes between the Sertoli cells are small and identical. Consequently I am not at all sure of tracing a Sertoli cell from one section to another. I tried staining lightly with Delafield's haematoxylin or haemalum sections varying in thickness from 6-24 micra. The spermatozoa however take the stain so lightly that they cannot be distinguished when packed in the cells. Sections fifteen micra thick stained with iron haematoxylin are so much better, that I made some counts from them, also from a section of tissue preserved in Flemming's fluid, but which I did not prepare. Thinking that there were twelve spermatozoa in each Sertoli cell, and that I might

not always have complete cells, in 15 micra sections, I tabulated only the larger numbers. The following are the counts:

11 Sertoli cells with 12 spermatozoa

18 " " " 11 "

20 " " " 10 "

In another effort to trace Sertoli cells from one section to another, I outlined several successive cross sections of tubules of 15 micra sections, located the groups of spermatozoa and counted them. Even though I did not succeed in tracing the cells, there were other results from these counts. Since Sertoli cells are about 50 micra long and 12 micra thick when fully developed, there should be in sections 15 micra thick, some complete cells, or cells with 12 spermatozoa, about the same number of cells with 11 and 1, with 10 and 2, with 9 and 3, with 8 and 4, with 7 and 5, and about twice as many with 6 spermatozoa, providing there are 12 spermatozoa in each Sertoli cell. But the following are the numbers in 6 sections:

14 groups of 1 each

26 " " 2 "

32	groups	of	3	each
32	"	"	4	"
57	"	"	5	"
70	"	"	6	"
65	"	"	7	"
35	"	"	8	"
13	"	"	9	"
6	"	"	10	"
2	"	"	11	"
3	"	"	12	"

Since this did not give any definite ratio, groups in eight sections 8 micra thick were counted, with the following results:

29	groups	of	1	each
24	"	"	2	"
27	"	"	3	"
26	"	"	4	"
49	"	"	5	"
44	"	"	6	"
41	"	"	7	"
21	"	"	8	"
6	"	"	9	"
3	"	"	10	"
0	"	"	11	"
1	"	"	12	"

In the first of these counts the sections were thick enough so that one Sertoli cell would not be in more than two sections, but the numbers of groups of 1, 2, and 3 spermatozoa far surpassed the numbers of groups of 9, 10 and 11. From this I infer that the number cannot always be as high as twelve, and seldom, if ever, higher. In the 8 micra sections, it would be possible

for a Sertoli cell to be in three sections, and this would account for a larger number of small groups of spermatozoa, but hardly for as great a difference as there is. In both counts the numbers of groups of 5, 6, and 7 are far in excess of other numbers.

10 Short pieces of unstained tubules were teased and stained. Some pieces of tubules were stained in alum cochineal, or Delafield's, cleared in cresote and then teased or cut longitudinally and spread out. One difficulty here is in being unable to determine the stage of development of the spermatozoa. It is quite seldom too that the Sertoli cells are sufficiently isolated to enable one to make counts of their contents. No counts were recorded but in those cells which seem to be complete there are more often 8, 9 or 10 spermatozoa, than 11 or 12.

// Finally, sections. 20, 25, 30, 35, and 40 micra thick were prepared and stained with iron haematoxylin with or without eosin, and with tricolor. The tricolor does not give as good results, though less destaining would probably improve it. With iron haematoxylin, the spermatogonia, spermatocytes and spermatids are all a light brown, and the spermatozoa a dead black when mature. Thus especially with the immersion oil objective, the spermatozoa

are clear and distinct. In the 20 micra sections a few Sertoli cells can be found, the spermatozoa of which are invisible at both the upper and lower plane of the section. In sections 30 micra thick a Sertoli cell with 7 or 8 spermatozoa may be in focus at the upper level of the section, disappear, and at the lower level another cell immediately beneath the first and equally well filled comes into focus. In 40 micra sections a third cell may sometimes be seen directly above two others. Thus there can be no doubt but what complete cells are contained in these sections. In all counts I was careful to have the complete thickness of the cell, and in practically all the entire length could also be traced. Counts were made in several stages. The clearest and easiest were those made when the spermatozoa were approaching and at the base of the cell, or at the base and beginning to move outward. In the latter case sections with the length of the lumen extending across the entire field, with the low power, were used and no spermatozoa were seen in the lumen, or had even reached the inner portion of the Sertoli cell. Counts of very early stages of spermatozoa were more difficult, because they are less clearly outlined, and the Sertoli cells are thicker, more

branching, and come in contact with each other about the lumen, thus making the groups of immature spermatozoa less distinct than the mature groups. The spermatids are not sufficiently grouped to give reliable accounts.

12 In all the following counts I was careful to focus each time to see that I had a complete cell. I first counted mature spermatozoa (Fig I & II) in longitudinal sections of tubules, in the lumens of which there were no spermatozoa; neither had the spermatozoa advanced to the inner portion of the Sertoli cells. Thus I am sure that none have escaped. The following groups of spermatozoa in Sertoli cells were counted in sections 30, 35 and 40 micra thick:

10	groups	of	6	spermatozoa
30	"	"	7	"
45	"	"	8	"
35	"	"	9	"
3	"	"	10	"

13 In sections of the same thickness I counted groups of spermatozoa which had just reached or were approaching the bases of the Sertoli cells, and obtained the following results:

9	Groups	of	6	spermatozoa
26	"	"	7	"
24	"	"	8	"
16	"	"	9	"
5	"	"	10	"
4	"	"	11	"

Finally I counted very early spermatozoa (Fig III) in sections 40 micra thick, most of which were stained with iron haematoxylin and counterstained with eosin. The counts of these immature spermatozoa as follows:

10	groups	of	6	spermatozoa
23	"	"	7	"
27	"	"	8	"
25	"	"	9	"
10	"	"	10	"
2	"	"	11	"
1	"	"	12	"

14 The total of 313 counts from sections 30, 35, and 40 micra thick are:

29	groups	of	6	spermatozoa
77	"	"	7	"
106	"	"	8	"
76	"	"	9	"
18	"	"	10	"
6	"	"	11	"
1	"	"	12	"

In two cells which appeared to be whole I could make out only 5 spermatozoa, and a few times, especially among immature spermatozoa, groups were so close together as at first to appear to form one, thus forming groups of more than twelve. It is possible that groups of 5 or 13 or 14 do occur, but if so, they are very rare. From these counts and the earlier ones I conclude that

the number of spermatozoa to each Sertoli cell varies from 6 to 12 and is oftener 7, 8 or 9.

15 The rod or crystal which Montgomery and Winiwarther describe is not visible in the human material which I have, but this can doubtless be accounted for in the fixation. The Sertoli cell nuclei stain dark, and are always irregularly shaped and distributed. The Sertoli cells of the cat testes on the contrary do show rods and rodlets, in the tissue preserved in Tellyesniczky's fluid as well as that preserved in Flemming's fluid. These rods are, I suppose, structures similar to those of the human Sertoli cells for they are long and slender, sometimes curved, and irregularly placed. They are, however, not as distinct as those of the human testis as illustrated by Montgomery and Winiwarther. It is only with very careful focusing that they can be seen at all, and a few times this focusing has made them appear to be corners or edges of the nuclear membrane. With their irregularly shaped nuclei this might indeed be possible. Winiwarther, although, he calls these structures crystals, and has discussed them at some length has been unable to prove their crystalline properties by polar-

ization of light and even says that he has never been able to identify with certainty these crystals of Charcot. Besides the rod or crystal both authors describe rodlets or accessory strips (Bâtonnets), which Montgomery says persist longer, while Winiwarther says the rods persist after the rodlets disappear. I have noted the rods after the Sertoli nucleus was well pushed away from the wall of the tubule of the cat testis.

16 None of the mature Sertoli cells of the rat testis show either rods or rodlets. About the periphery of a section preserved in Fleming's solution and stained with iron haematoxylin there are occasional dark streaks on the Sertoli nuclei, which I at first thot were rods. These are not visible in the lighter cells of the central part of the section. The nuclei are uniformally slightly granular and contain a large nucleolus and two or three smaller chromatic bodies, one always close to or attached to the nucleolus.

17 In the human testis by far the greater part of the cells seen were primary spermatocytes in various stages, but none dividing. At the periphery there were also a few cells in earlier stages, and at the lumen some spermatids and spermatozoa. The fact that so many

are in the same condition would seem to show that there are only certain times when there are mature spermatozoa and not continuously as in the rat. Some sections of tubules of the rat testis show a row of developing primary spermatocytes at the periphery, then secondary spermatocytes between the long Sertoli cells which contain mature spermatozoa. Other tubules show the primary spermatocytes almost mature, secondary spermatocytes, and Sertoli cells with spermatozoa, pulling away from the wall of the tubule and passing toward the lumen. At the same time there can be seen in other tubules a row of the large primary spermatocytes inclosing a mass of secondary spermatocytes which in turn enclose the last of the escaping spermatozoa. The secondary spermatocytes divide quickly into spermatids, and then first begin to form in groups. When they are seen elon gated and in definite groups about the inner ends of the Sertoli cells which have grown inward the mature primary spermatocytes are dividing and the next generation is beginning to appear. The spermatozoa begin to form at the periphery of the lumen, but as they condense more and as the secondary

spermatocytes which are being formed, push outward, these spermatozoa make their way toward the base of the Sertoli cell, often clustering close about the nucleus. Even when passing out the nucleus is frequently very close to the spermatozoa.

Montgomery in speaking of ultimate spermatogonia one quarter of which he claims contain rods and three quarters lack rods, says: "The ratio is somewhat less than 1:3, which is readily explained on the ground that some of the spermatogonia with rods had already become Sertoli cells and therefore were not included in the count". It would seem then that Sertoli cells develop from the ultimate spermatogonia, before the other three cells divide and develop into primary spermatocytes. Then there ought to be Sertoli cells, perhaps not quite mature, for each generation of germ cells in the tubule. Usually three generations of germ cells- primary and secondary spermatocytes, and spermatozoa,-are distinctly visible in the tubules. But only when one generation of spermatozoa and Sertoli cells are passing out can a second generation of Sertoli cells be distinguished. Where, then, are the successive quarters of ultimate spermatogonia which contain

the rod, some of which develop into Sertoli cells before the other three quarters of ultimate spermatogonia can divide? It would seem that they would at least be clearly visible by the time the other three quarters had become secondary spermatocytes.

Conclusions:

1. Spermatogenesis is continuous in the rat, mature sperm being found at all times, but it is not continuous in man.

2. The Sertoli cells of the rat do not show a rod like structure, but those of the cat do.

3. There is no definite connection between Sertoli cells and germ cells until the spermatozoa are beginning to form.

4. The number of spermatozoa to each Sertoli cell varies from 6 to 12 in the rat.

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These drawings were made at the level of the stage with a camera lucida. Ocular 8 was used for all.

Fig I was drawn with the 16 mm objective.

Fig II & III with the 18 mm immersion oil objective.

Fig I 40 micra section of a seminiferous tubule of the rat, showing spermatozoa at the bases and starting out from the bases of the Sertoli cells.

Fig II Part of the same from a 20 micra section more highly magnified.

Fig III 20 micra section showing the spermatozoa forming.

a Sertoli cell

l Lumen of tubule

n Nucleus of Sertoli cell

s Spermatozoa

sg Spermatogonia

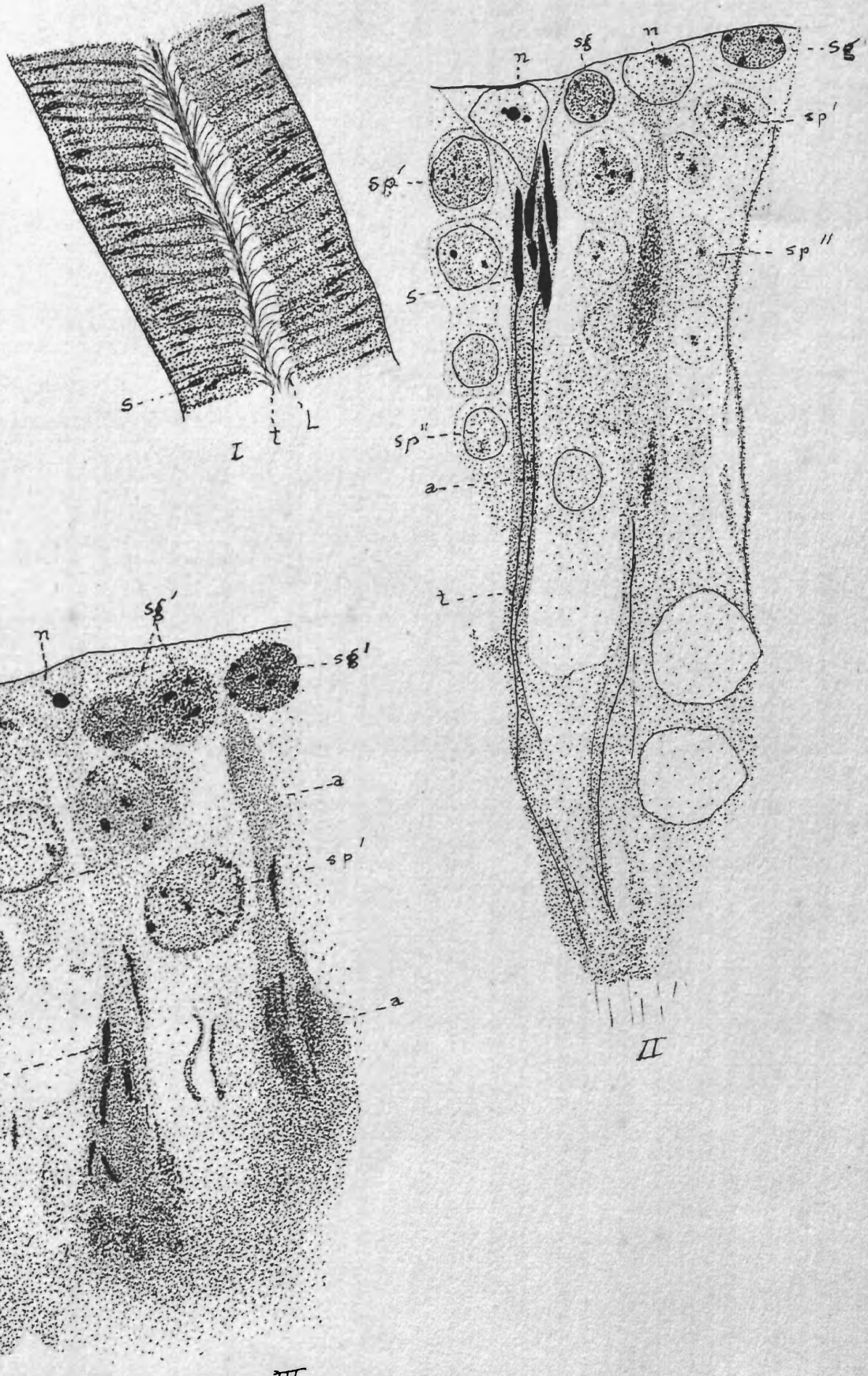
sg' Spermatogonia growing into primary spermatocytes.

sn Part of spermatogonial nucleus not in focus

sp' Primary spermatocytes

sp" Secondary spermatocytes

t Tail of spermatozoa.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