später einmal sicher wiederzuerkennen. Darüber hinaus glaube ich aber auch, daß die vorliegende Form mit der vor 44 Jahren beschriebenen und bisher nicht wiedergefundenen *Oithona hebes* GIESBRECHT gleichgesetzt werden kann. Daß meine Tiere mit den GIESBRECHTschen hinsichtlich des gegenseitigen Längenverhältnisses von Vorder- und Hinterkörper nahezu identisch sind, wurde bereits erwähnt. Ebenso gut ist die Übereinstimmung nach meinem Dafürhalten aber auch in allen anderen vergleichbaren Merkmalen, wie Beschaffenheit des Analsegmentes, der Furkaläste, Bau der Stirn und Länge der Vorderantennen. Wenn schon die hier in Frage stehende Art mit einer bereits beschriebenen identifiziert werden soll, so kommt meines Erachtens keine andere Form mehr in Betracht als eben *Oithona hebes*.

Mit der SCOTTschen *minuta* kann *hebes*, wie sie hier aufgefaßt wird, auf keinen Fall gleichgesetzt werden, wie das ROSENDORN vermutet hat. Denn beide unterscheiden sich u. a. grundlegend dadurch, daß bei jener das Glied des rudimentären Füßchens mit zwei Anhängen versehen ist (= Gattung *Dioithona mihi*, in: Zool. Anz. **112**, 322), während hier bei *hebes* wie bei den meisten Oithonen nur eine Borste vorhanden ist.

A Cytological Study of Macrostomum tuba von Graff.

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(With 25 Figures.)

Eingeg. 5. März 1936.

Although the Rhabdocoela present an exceptionally favorable group of animals for a chromosome study, comparatively few chromosome numbers have been reported for this order. The results obtained from cytological studies, when used as taxonomic characters in addition to those already employed, should be valuable in solving many of the problems of systematic position that exist within this group. An application of the results obtained in this manner is in accord with some of the more recent developments in taxonomic work.

The cytological studies of Rhabdocoela already completed show that the members of this group possess very low chromosome numbers. HARVEY (1920), covering the years 1878—1918, tabulated the reported chromosome numbers of this order. SENN (1935) found the chromosome number for *Olisthanella virginiana* KEPNER and CARTER to be $n = 2$, $2n = 4$. His paper includes a brief review of the cytological investigations of the Rhabdocoela.

RUEBUSH (1935) reported the diploid number of *Provortex affinis* JENSEN to be six. He states that apparently one may recognize three morphologically distinct pairs of chromosomes. The present paper reports the number for *Macrostomum tuba* von GRAFF to be $n = 3$, $2n = 6$.

The following is a complete list of reported chromosome numbers of Rhabdocoela:

Species	Family	n	$2n$	Authority
Graffilla gemellipara	Graffillidae	4	8	PATTERSON, 1912
Paravortex gemellipara . . . (*Graffilla gemellipara*) . .	,,	4	8	BALL, 1916
Paravortex cardii	,,	2	4	HALLEY, 1908
Vortex viridis . . . . . (*Dalyellia viridis*)[1]	Dalyelliidae	2	4	LEPESCHKIN, 1910
Mesostomum ehrenbergi . .	Typhloplanidae	—	ca. 7	SCHNEIDER, 1883
,, ,,	,,	5	10	BRESSLAU, 1904
,, ,,	,,	5	—	LUTHER, 1904
Mesostoma ehrenbergi . . . . (*Mesostomum ehrenbergi*)	,,	5	10	VON VOSS, 1914
Mesostomum lingua	,,	3	—	LUTHER, 1904
Olisthanella virginiana . . .	,,	2	4	SENN, 1935
Provortex affinis	Provorticidae	—	6	RUEBUSH, 1935
Macrostoma hystrix . . . . . (*Macrostomum hystrix*) .	Microstomidae	2	—	LUTHER, 1905
Macrostoma viride . . . . . (*Macrostomum viride*) . .	,,	2	—	LUTHER, 1905
Macrostomum tuba	,,	3	6	PHILLIPS, 1935

It is known that the chromosome numbers may differ widely within large groups and even within species. Nevertheless, the fact is not to be overlooked that some groups show characteristics peculiar with respect to chromosome numbers and morphology. In some cases a given number or a peculiar type of chromosome complement exists throughout a group. The genera of Rhabdocoela apparently display these characteristics. The recorded diploid numbers of the order Rhabdocoela show a range of from four to ten. Eight species representing five families have been studied. It is rather interesting that this large group, which includes very diverse types of species, should exhibit such a limited range of chromosome numbers. The small range may indicate close phylogenetic relationship within the order.

Macrostomum tuba VON GRAFF is a flatworm of the family Microstomidae. The American animal, which appears to be a variety of the European species, was described by KEPNER and STIFF (1932). The specimens found at that time differ from the European representatives in but one essential particular, the

[1] HEIN (1928) reported the diploid chromosome number of *Dalyellia viridis* to be four. This number should be included in the above list. HEINS investigation was encountered after this paper had gone to press.

penis. Since the gross anatomy has been adequately described further anatomical evidence is unnecessary. The purpose of this paper is to describe spermatogenesis and report the number and morphology of the somatic and meiotic chromosomes. The members of this genus are favorable for chromosome studies not only because of their abundance and adaptability to culturing, but especially because of their small chromosome numbers.

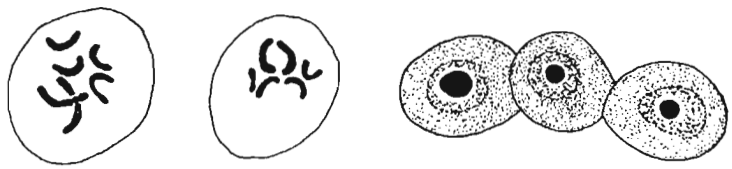
The animals were collected near the University of Virginia, placed in Stender dishes, and kept until sexually mature. These flatworms were fed about twice weekly on fragments of annelids and of tadpole liver. They may be cultured for an unlimited length of time in this manner.

GOLDSMITH'S, chrom-aceto-formalin, BOUIN'S, and ZENKER'S fixing fluids were used. GOLDSMITH'S and hot ZENKER'S proved to be most effective.

Frontal, saggital, and transverse sections were made at five and seven μ. HEIDENHAIN'S iron-hematoxylin method was used as a staining process.

All drawings were made with a camera lucida and at a magnification of $\times$ 2000. A Zeiss microscope, equipped with a $\times$ 15 ocular and a $\times$ 90 objective n. a. 1.25, was used.

The diploid chromosome number of *M. tuba* is six. A study was made of the somatic chromosomes of mesenchymal cells, oogonia, and spermatogonia. The mesenchymal cells showed comparatively few divisions, but the metaphase chromosomes of



1. 2. 3.

Fig. 1. Somatic mitosis showing chromosomes of a mesenchymal cell, $2n = 6$.
Fig. 2. Metaphase plate of spermatogonium.
Fig. 3. Resting nuclei of spermatogonia.

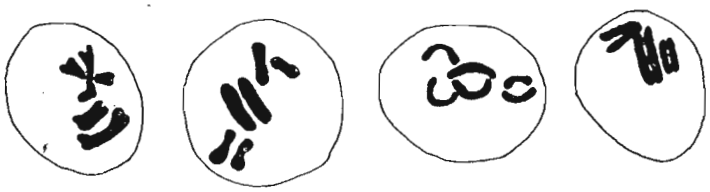
these divisions were well spaced and well defined (Fig. 1). A comparison of the positions of the chromosomes in a number of mesenchymal metaphases shows that the arrangement varies greatly. The chromosomes of the spermatogonia are somewhat smaller and more compact than those of either the mesenchymal cells or oogonia (Fig. 2). There is a striking similarity between the spermatogonial and meiotic chromosomes. The resting nucleus of a spermatogonium possesses a large, deeply staining nucleolus around which a clear space, or non-staining area, is observed (Fig. 3). The somatic cells possess one pair of large, one pair of medium, and one pair of small chromosomes; all of them have median attachment constrictions. The small size of the chromosomes prohibits a detailed description of their morphology.

The haploid chromosome number of *M. tuba* is three. The spermatogonia enlarge somewhat to form primary spermatocytes. At the beginning of the prophase of the first division, the six leptotene threads are distributed, apparently without any special orientation, throughout the nucleus. These threads possess small chromomeres which are spheroidal in contour (Fig. 4). No split chromomeres were observed prior to synapsis. The nucleolus does

4. 5. 6. 7.

Fig. 4. Formation of leptotene threads with spheroidal chromomeres.
Fig. 5. The zygotene stage showing the synapsis of homologous chromosomes. Each separate thread possesses chromomeres.
Fig. 6. Pachytene.
Fig. 7. Diplotene. The chromomeres are obscured by the presence of extra chromatin.

not persist after the leptotene stage. The leptotene threads conjugate in homologous pairs to form the zytogene stage (Fig. 5). At this stage no well defined matrix was observed. The three closely paired threads become slightly thicker and more compact to form the pachytene threads (Fig. 6). The pachytene then passes into the diplotene stage, and as the result of the accumulation of extra chromatin material around the chromonemata, the separate



8. 9. 10. 11.

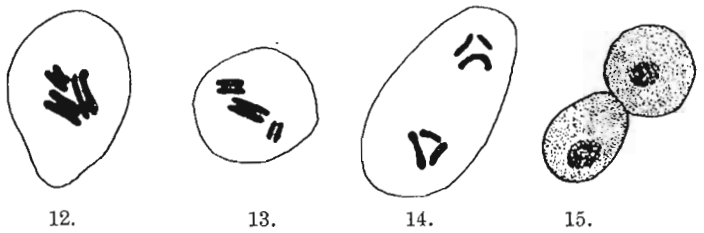
Fig. 8. Diakinesis.
Figs. 9 and 10. Metaphases of first maturation divisions, spindle views, $n = 3$.
Figs. 11. Anaphases of first maturation divisions.

threads are obscured (Fig. 7). The chromosomes become short and thick, and, at diakinesis, the three pairs lie scattered throughout the nucleus (Fig. 8).

The synaptic pairs of chromosomes arrange themselves in a close association to form the first metaphase plate (Figs. 9 and 10). The anaphases of this first division show the dyads very plainly (Figs. 11 and 12). No metabolic nuclei are formed as a result of interkinesis; the chromosomes persist as such and pass directly to second metaphase with only a slight change in form (Fig. 13).

The chromosomes of the second division are slightly smaller and more slender than those of the first division (Fig. 14).

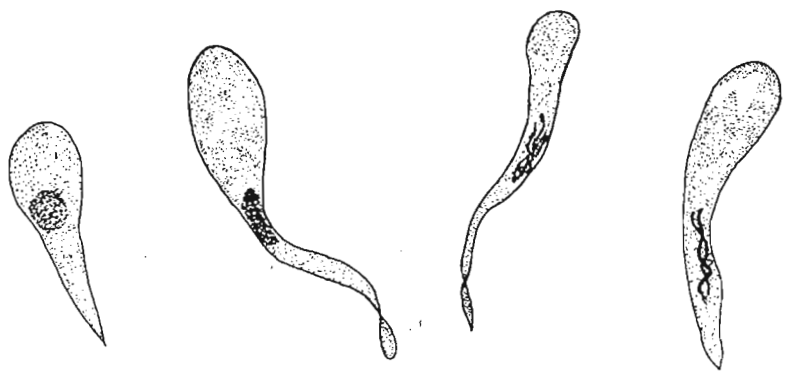
The secondary spermatocytes, which result from the first division, give rise to spermatids with granular resting nuclei (Fig. 15). When the cell membrane and cytoplasm of the spermatid



12. 13. 14. 15.

Fig. 12. Anaphases of first maturation divisions.
Fig. 13. Metaphase of second maturation division, $n = 3$.
Fig. 14. Anaphase of second maturation division.
Fig. 15. Spermatids with resting nuclei.

elongate to form the tail-piece of the spermatozoon, the nucleus takes up a peripheral position close to the cell membrane and near the elongated portion (Fig. 16). As the tail-piece becomes more elongated, the nucleus moves into it (Fig. 17). At this stage the nuclear membrane breaks down, and the chromatin material



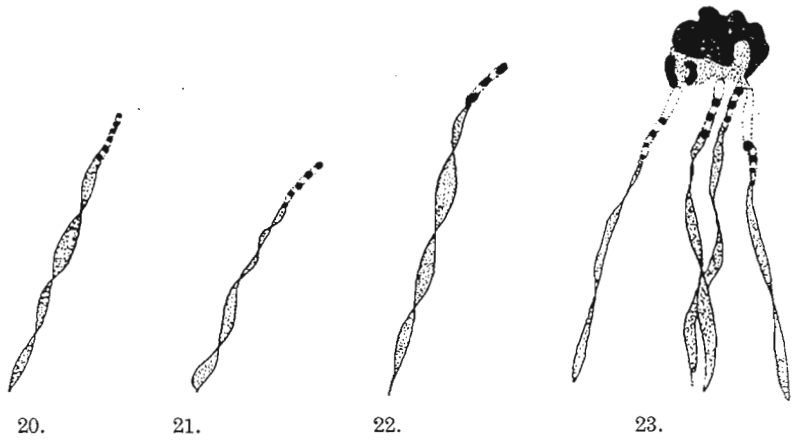
16. 17. 18. 19.

Fig. 16. Elongation of spermatid cell membrane and cytoplasm to form tail-piece of spermatozoon. Movement of nucleus into tail-piece.
Fig. 17. Disintegration of nuclear membrane.
Figs. 18 and 19. Formation of chromonemata.

takes the form of chromonemata (Figs. 18 and 19). During the early formation and development of the chromonemata, the threads appear diffuse and irregular. The accumulation of chromatin material around the chromonemata produces distinct, darkly staining threads. These form six distinct chromatin granules (Fig. 20). The granules move forward to form the head-piece of the spermatozoon. Several hundred spermatozoa have been studied; each of them possesses either six, five, four, or three

granules (Figs. 20, 21, 22, and 23). This variation in numbers suggests that adjacent granules have become closely associated. Further evidence for this conclusion is derived from the fact that when three granules are present, each of them is larger than a single granule of the group of six. The granules were observed when either of the fixatives was used.

During their development, some of the spermatozoa become associated with accessory or nurse cells. These cells apparently play an important part in the nutrition of the spermatozoa. The connection is established after the spermatozoon has been formed and at the time the chromatin granules become differentiated (Fig. 23). The staining intensity of these nurse cells in the fixed specimens shows that the cells contain a large amount of material



Figs. 20, 21 and 22. Spermatozoa possessing six, five, and three granules.
Fig. 23. A nurse cell with attached spermatozoa.

that may represent food. It is impossible to differentiate between the nurse cells and the spermatogonia during the early development of each group. Both may pass through mitotic stages of division, but there is no indication that the spermatogonia and nurse cells pass through their subsequent stages of development as associated units. Each shows separate and individual development. Apparently the nurse cells are modified spermatogonia which, by a physiological division of labor, contribute to the development of the sperm. A single nurse cell acts as an anchorage for from one to five spermatozoa which it may nourish, four and five being most frequently found. The heads of the spermatozoa become partly imbedded in, and firmly attached to, the cytoplasm of the nurse cell. The spermatozoa remain attached in this position until they mature; then they are released and pass from the lobules of the testes into the vasa deferentia. Unattached

spermatozoa are also found in the testes. The number of nurse cells, in comparison with the number of spermatozoa, is very small; many of the spermatozoa evidently derive nourishment from the surrounding fluids.

Oogonial divisions have been observed, but such divisions in *M. tuba* are rare. Very often sexually mature animals do not show a single division. The metaphase plates of these divisions show six chromosomes that are morphologically similar to the

Fig. 24. Somatic mitosis showing metaphase chromosomes of an oogonial division.

chromosomes of the spermatogonia (Fig. 24). The reduction division has not been observed in the ovaries of the specimens studied.

During the development of the oogonium, it is uniformly filled with granules. These granules later become differentiated

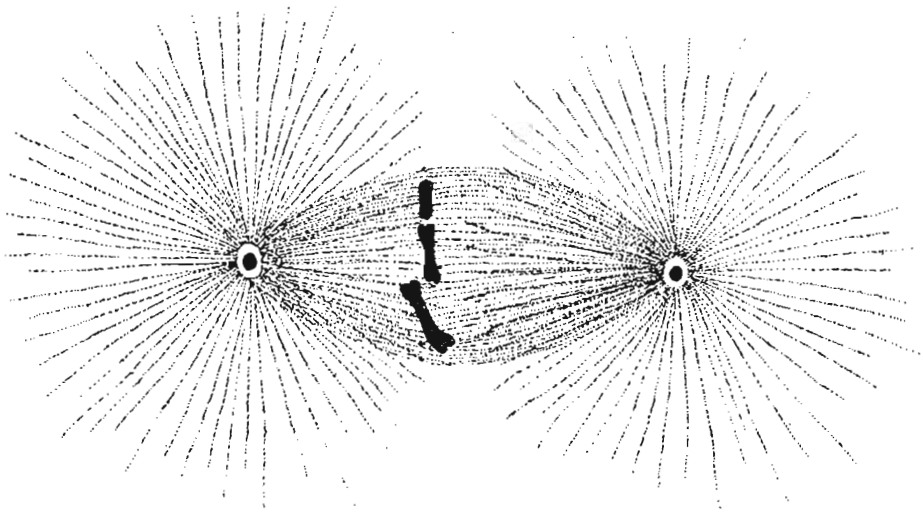


Fig. 25. Large spindle bearing three chromosomes observed in the egg as it travels down the oviduct.

into peripheral ones, which will probably take part in shell formation, and into deutoplasmic granules.

Sections of the egg fixed either at the time it migrates down the oviduct, or after it has reached the genital atrium, show a very large division spindle. Three chromosomes are orientated upon the equatorial plate of this spindle (Fig. 25). The nature of the division has not been definitely determined. It was suggested by BARRETT (1930) that in *Macrostomum appendiculatum* this division represents the first cleavage of a parthenogenetically

developing egg. But, the structure of the chromosomes and the fact that no reduction divisions were observed in the ovaries indicate that the division is a maturation metaphase. The apparent non-occurrence of haploid individuals also suggests that parthenogenesis does not occur, but it is possible that diploidy is established by a division of the chromosomes without a subsequent cell division. A further study, upon animals in all stages of sexual development, will be made at this laboratory in order to interpret adequately these observations.

The origin and subsequent development of the chromatin granules found in the heads of the spermatozoa is of such unusual occurrence as to warrant further investigation. A study of them throughout the complete life history of the animals would be desirable. A review of the literature shows that very little is known concerning the reproduction of *M. tuba*, and such a study was not included in the present work. In *Macrostoma tuba*, LUTHER (1905) observed the presence of three chromatin granules in the heads of the spermatozoa. Apparently the granules were formed from chromatin threads.

If the three chromatin granules were found to act as individual chromosomes after the egg has been penetrated by the spermatozoon, it could be assumed that the six chromatin granules represent the split or double chromosomes formed by the double chromonemata. On this assumption, the chromosomes of the spermatozoa would be double in nature. PATTERSON (1933) found that the x-raying of the mature sperm cells of *Drosophila* produced one mosaic female in every seven females. This indicates that at the time of treatment the X-chromosome is split in about one out of every seven spermatozoa.

MUSLOW (1911) described the nematode *Ancyracanthus cystidicola* as a form in which the chromosomes of the sperm remain separate and distinct. They may be readily counted even in the living spermatozoa. Since all of the fixatives used produced the same results in *M. tuba*, it seems hardly plausible that the chromatin granules in the fixed specimens were artifacts caused by a specific fixative. The granule-like structures are visible in the living spermatozoa, but it is impossible to trace their stages of development in the living state.

Nurse cells have been described for other animals, but no description of a nurse cell occurring in the order Rhabdocoela has been encountered.

I wish to acknowledge the helpful criticisms and suggestions of Dr. WILLIAM A. KEPNER, who classified the animals and directed this investigation.

Summary.

1. The members of the genus *Macrostomum* are very favorable for cytological studies.

2. The haploid and diploid chromosome numbers of *M. tuba* are three and six, respectively.

3. The chromosome complement consists of one large pair, one medium pair, and one small pair of chromosomes; all of them have median attachment constrictions.

4. The spermatozoa possess from three to six chromatin granules, the members present depending upon the number of paired, adjacent granules which have become so closely associated that they appear as one granule; it is suggested that each individual granule represents a chromatid.

5. At the time the granules are formed, the spermatozoa may become associated with accessory or nurse cells. These cells have their origin as spermatogonia. A single nurse cell may serve as an anchorage for from one to five spermatozoa.

6. The oogonial divisions show six chromosomes.

7. A large spindle bearing three chromosomes is present in the developing egg and apparently represents a maturation division.

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