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# Comparative Observations on the Development of Germ Cells between *Paragonimus westermani* (Kerbert, 1878) and *P. pulmonalis* (Baelz, 1880)

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Recently, Miyazaki (1977, 1978a, 1978b) proposed that so-called *Paragonimus westermani* (Kerbert, 1878) would be devided into two types by the presence or absence of spermatozoa in the seminal receptacle. One is *P. westermani* (s. str.) that was described by Kerbert and has numerous spermatozoa. The other has no spermatozoon and is regarded as *P. pulmonalis* (Baelz, 1880) which has been rejected as a synonym of *P. westermani*.

Prior to the above, several cytological studies were made on so-called *P. westermani*. Sakaguchi and Tada (1976) and Terasaki (1977) revealed that *P. westermani* was triploid and had a very few, if any, spermatozoa in the testes. No figure of meiotic division was found in any germ cell in ovaries and testes. It is considered that the lung fluke carries out parthenogenesis. Cho *et al.* (1977) pointed out the abnormal gametogenesis and fertilization of *P. westermani* and suggested that it should perform parthenogenesis. All of *P. westermani* used in the above studies were collected in Kyushu district, Japan. According to Miyazaki (1978b), *P. westermani* occurring in Kyushu belongs to *P. pulmonalis*.

Terasaki (1978) analyzed the karyotype of *P. westermani* collected from Akita Prefecture and confirmed that those flukes were diploid (2n=22). Miyazaki (1978b) referred that the fluke in Akita is *P. westermani*. Further, Terasaki (1979) conducted the analysis of lung fluke from Kangwon-Do, Korea, and confirmed that these flukes were triploid. Miyazaki (1978b) stated that these flukes in Kangwon-Do were *P. pulmonalis*.

*P. pulmonalis* proposed by Miyazaki is not totally accepted as an independent species. However, the author agrees with Miyazaki's opinion and deals with *P. pulmonalis* and *P. westermani* as different species as far as there is no evidence found to deny his opinion up to now.

By the fact of the absence of spermatozoon in seminal receptacle, and of triploid chromosomes in *P. pulmonalis*, it is presumed that the spermatogenesis in *P. pulmonalis* might be disturbed in the process of cell division. In the present investigation, the comparative study of germ cells between *P. westermani* and *P. pulmonalis* was made by using a phase contrast and a light microscopes.

## Materials and Methods

The materials used are shown in Table 1. Metacercariae of *P. westermani* were removed from *Geothelphusa dehaani* collected at Nishiki-mura, Semboku-gun, Akita, Japan. Those of *P. pulmonalis* were obtained from *Cambaroides similis* collected at Uhseongjeon-Ri, Yangyang-Kun, Kangwon-Do, Korea. The metacercariae of both species

Table 1 The origin and experimental obtaining of the lung flukes, *P. westermani* and *P. pulmonalis*

| Lung flukes | Intermediate host | | Experimental inoculation | | | Purpose of observation | No. of flukes used |
|---|---|---|---|---|---|---|---|
| | Species | Localities | Host | Method | Duration (days) | | |
| *P. westermani* | *Geothelphusa dehaani* | Nishiki-mura, Semboku-gun, Akita, Japan | Dog | Oral | 135 | For nucleus | 3 |
| | | | Dog | Oral | 175 | For nucleus | 3 |
| | | | | | | For cell body | 3 |
| | | | Cat | Abdominal | 186 | For nucleus | 6 |
| | | | | | | For cell body | 3 |
| *P. pulmonalis* | *Cambaroides similis* | Uhseongjeon-Ri, Yangyang-Kun, Kangwon-Do, Korea | Dog | Oral | 70 | For nucleus | 7 |
| | | | Cat | Abdominal | 182 | For nucleus | 10 |
| | | | | | | For cell body | 3 |
| | | | Cat | Abdominal | 226 | For nucleus | 13 |
| | | | | | | For cell body | 3 |

were separately administered to dogs orally and/or cats by abdominal injection. These infected animals were sacrificed several months after the administration, and mature lung flukes were recovered from the worm cysts in their lungs.

Twelve worms of *P. westermani* and 30 of *P. pulmonalis* were prepared by air-drying method (Terasaki, 1977, 1978) so that the nuclei of germ cells might be observed. These specimens were stained by Giemsa solution and observed by a light microscope.

Each six worms of both species were prepared as follows: The ovary, testes, and seminal receptacle were removed from the worms by the aid of the dissecting microscope and placed on slide glasses separately. One or two drops of physiological saline were put on each organ. The ovary and testes were broken with a micropin to disclose germ cells and were spread to an appropriate extent. The seminal receptacle was covered with a cover glass without any treatment. Observation was performed by a phase contrast microscope (Model BHB, Olympus Optical Industry Co., Ltd.).

## Results

*Living germ cells in* P. westermani

In observing *P. westermani* using a phase contrast microscope, various stages of spermatogenesis from spermatogonia to spermatozoon were seen (Fig. 1). In the testes, the spermatogonium before division was present, and 2-, 4-, 8-, 16-, and 32-cell masses in the process of division were also seen. The cells in each mass were connected with one another by specific intercellular bridges and they formed a structure, so-called "rosette" (Figs. 3-7). In some rosettes of 32-cell masses, the free ends of the cell exterior to the cytophore were slightly projected out, and there were two flagella extending from both sides of the projections (Fig. 8). Some projections and their two flagella were thrusted further to form three processes (Fig. 9). Some of the cells with long projections formed a rosette, and were in the process of decomposition (Fig. 10). In the stage, the flagella were fused with cellular projections in almost all portions.

Numerous spermatozoa were observed in the seminal receptacle of *P. westermani* (Fig. 17). Some of them looked like long filaments, and some had bifurcated tips (Fig. 18).

The cells in the ovary did not form such rosettes as observed in the testes (Fig. 19).

*Nuclei of germ cells in* P. westermani

In specimens of testes prepared by the air-drying method, on the contrary to the observation by a phase contrast microscope, nuclei of germ cells in interphase of cell

division, chromosomes on cell division, and nuclei of spermatids in spermiogenesis were noticed under a light microscope (Fig. 21).

The nuclei of germ cells in the process of cell division formed masses of 2-, 4-, 8-, 16-, and 32-nuclei (Figs. 22–26), which seem to correspond to each "rosette" observed in the living germ cells under a phase contrast microscope. The sizes of the nuclei of 32-nucleus masses were somewhat smaller than those of the other nucleus masses (Fig. 26). Some of the nuclei of 32-nucleus masses were small and condenced (Fig. 27).

Both meiotic and mitotic figures in the metaphase of cell division were seen. Some of the figures have 11 or 22 chromosomes, and some have the multiple number of these chromosome numbers, which seems to be orthoploidy.

Each mass of the nuclei of spermatids on spermiogenesis consisted of 32-nuclei. The transformation was slight in some masses (Fig. 28) but was much progressed in other masses (Figs. 29, 30).

In observing ovary, the nuclei of germ cells were separately observed. They did not form masses like the nuclei seen in the testes. The meiotic chromosome number was 11, and mitotic one was 22.

*Living germ cells in* P. pulmonalis

In the observation on the living cells of testes of *P. pulmonalis*, the process of spermatogenesis was similar to that found in *P. westermani*, up to the stage of the formation of 32-cell masses. The testes of *P. pulmonalis* included, however, neither spermatozoa nor cells in the process of spermiogenesis (Fig. 2). Two- and 4-cell rosettes were relatively small in number (Figs. 11, 12). On the other hand, while 8-cell rosettes were abundantly found (Fig. 13) and followed by 16-cell ones in frequency (Fig. 14), only a few 32-cell rosettes (Fig. 15) which were composed of cells with their external free ends flattened and blunted were found. These 32-cell rosettes were lacking of cellular projections and flagella as recognized in *P. westermani* (Fig. 16).

In observation of the seminal receptacle, there were no spermatozoon but many round cells in it.

No difference was found in the ovary between *P. westermani* and *P. pulmonalis* (Figs. 19, 20).

*Nuclei of germ cells in* P. pulmonalis

In the observation on specimens of testes prepared by air-drying method, the nuclei of germ cells in the interphase stage of cell division and chromosomes were confirmed under a light microscope, instead of any nucleus of spermatid on spermiogenesis (Fig. 32). These nuclei of germ cells in the interphase of cell division consisted of groups of nuclei from 2- to 32-cells (Figs. 33–37) just as those of *P. westermani*. However, the nuclei of 32-cells were not condensed and their density was low (Fig.37).

In the metaphase stage of germ cells in testis, only mitotic univalent chromosomes were observed instead of meiotic bivalent ones. Their numbers were counted from 33 to their multiple numbers.

In observation of ovary, just as similar as that of *P. westermani*, each nucleus of germ cells was observed separately, however, the meiotic metaphase figure consisting bivalents was not noticed.

## Discussion

The morphology of spermatozoa and spermatogenesis in the Plathelminthes, especially in Trematoda, has been cleared in detail on many species both by a light microscope and an electron microscope (Burton, 1960, 1972; Grant *et al.*, 1976; Gresson and Perry, 1961; Kitajima *et al.*, 1976; Nez and Short, 1957; Otsuji *et al.*, 1976). As for the lung flukes in Japan, Sato *et al.* (1967) carried out studies on spermatogenesis in *Paragonimus miyazakii* Kamo et al., 1961, using the transparence electron microscope (TEM). Recently, Fujino *et al.* (1977) performed the same studies on *P. ohirai* Miyazaki, 1939, using the scanning electron microscope (SEM). From the results mentioned above,

at least the figure of the spermatozoon and the process of spermatogenesis in *P. ohirai* and *P. miyazakii* were confirmed to be essentially similar to each other and the spermatozoa of these species were found to perform the following process of development: A spermatogonium in the peripheral portion of the testis undergoes division three times to be transformed into eight primary spermiocytes. Then these cells undergo meiotic division twice to produce 32 spermatids. In these processes, the cells produced are not separated completely, but are connected with one another by intercellular bridges so as to form "rosettes" in every stage. In the 32-cell rosette, each free end of the cell on the outer side is soon thrusted to form a cellular projection. Two flagella are developed from both sides of each cellular projection, which extends further. The nucleus proceeds into the projection to reach its free end. At the same time the two flagella are fused with the cellular projection as the nucleus moves to the end of it. The projection fused with the flagella is separated from the cell body and develops into a long, slender spermatozoon.

In the present observation, all of each stage mentioned above were found in the testes of *P. westermani*, and numerous spermatozoa were included in seminal receptacle though about one-third of the spermatozoa observed had bifurcated ends (Fig. 18). Therefore, it is considered that the spermatogenesis of *P. westermani* is performed in the same manner as mentioned above.

No remarkable was found in the process of spermatogenensis up to the stage of 32-cell masses between *P. westermani* and *P. pulmonalis* in so far as observed in the present study. It is, however, considered that the manners of divisions of germ cells between *P. westermani* and *P. pulmonalis* are different because of the following facts: No meiotic figure was found in the gonad of the triploid flukes (*P. pulmonalis*) (Sakaguchi and Tada, 1976; Terasaki, 1977) or the chromosomes in the primary spermiocyte were completely asynaptic in the triploid flukes (Cho *et al.*, 1977), but the numerous meiotic figure were found in the gonad of diploid flukes (*P. westermani*) (Terasaki, 1978).

In the 32-cell stage of spermatogenesis of *P. pulmonalis*, almost of germ cell produced had a flattened end and was lacking of a cellular projection (Fig. 16), and no nucleus of spermatids on spermiogenesis was observed in the specimens prepared by air-drying method. However, cells similar to spermatozoon were present in a limited specimens when the testis was observed in such specimens as prepared by the squashing method (Terasaki, 1977; Cho *et al.*, 1977) or when the testis, vas deferens, and seminal vesicle were examined in mounted specimens of *P. pulmonalis* (Miyazaki, 1978a). Such cells seemed to be in the process of spermiogenesis, since nuclei of them were thicker and shorter than those of perfect spermatozoa. Therefore, it can not be concluded that no spermiogenesis takes place in *P. pulmonalis*. Eventually, it is presumed that in this species spermatogenesis may be interrupted by the time most germ cells reach the 32-cell stage and these cells do not undergo even condensation of chromatin, and that spermiogenesis may take place in a very few cells.

In the six flukes of *P. pulmonalis* observed in the present investigation, the seminal receptacle was found to contain a number of spherical cells but no spermatozoon. According to Miyazaki (1978a), this receptacle contains egg cells and vitelline cells, and very rarely juvenile eggs (Miyazaki, unpublished date).

There was no difference recognized in the ovary between *P. westermani* and *P. pulmonalis* except the number of chromosomes and the metaphase figure of spermiocyte.

The mixoploidy consisting of 2n and 3n, the quantitative variation of chromatin in the nuclei on the spermatozoa, and the inequality of cell sizes in the stages of 16-cell and 32-cell masses were not found in the testes of *P. pulmonalis* in so far as observed in the present study, though they were found in those of the common liver flukes in Japan

and Korea (Moriyama *et al.*, 1977, 1979; Kayano and Cho, 1978; Sakaguchi and Kusano, 1979; Moriyama and Terasaki, 1979).

## Summary

Using two species, *Paragonimus westermani* and *P. pulmonalis*, germ cells of their testes, ovaries, and seminal receptacles were observed by a phase contrast microscope. Testes and ovaries prepared by air-drying method were also observed. The results obtained are as follows: Spermatogenesis in *P. westermani* resembled that of other lung flukes (*P. ohirai* and *P. miyazakii*), while in *P. pulmonalis* almost all of spermatogonia might stop at the 32-cells stage (primary spermatid) without condensation of chromatin, and did not proceed to spermiogenesis. It seems that cell division from primary spermatocyte to spermatid in *P. pulmonalis* were mitotic (somatically meiotic) instead of being meiotic (Cho *et al.*, 1977). A large number of spermatozoa were observed in the seminal receptacle of *P. westermani*, whereas, in that of *P. pulmonalis*, only some circular cells were observed. No remarkable difference was seen between the ovarian germ cells of the two species except the number of chromosome and the figure of meiosis.

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### References

1) Burton, P. R. (1960): Gametogenesis and fertilization in the frog lung fluke, *Haematoloechus medioplexus* Stafford (Trematoda: Plagiorchiidae). J. Morphol., 107, 93–122.
2) Burton, P. R. (1972): Fine structure of the reproductive system of a frog lung fluke. III. The spermatozoon and its differentiation. J. Parasit., 58, 68–83.
3) Cho, H., Sasada, K. and Takao, Y. (1977): Gametogenesis of *Paragonimus westermani*. Chromosome Inf. Serv., (23), 29–30.
4) Fujino, T., Ishii, Y. and Mori, T. (1977): Ultrastructural studies on the sprmatozoa and spermatogenesis in *Paragonimus* and *Eurytrema* (Trematoda: Digenea). Jap. J. Parasit., 26, 240–255. (In Japanese with English summary)
5) Grant, W. C., Harkema, R. and Muse, K. E. (1976): Ultrastructure of *Pharyngostomoides procyonis* Harkema 1942 (Diplostomatidae). 1. Observations on the male reproductive system. J. Parasit., 62, 39–49.
6) Gresson, R. A. R. and Perry, M. M. (1961): Electron microscope studies of spermateleosis in *Fasciola hepatica* L. Expt. Cell Res., 22, 1–8.
7) Kayano, H. and Cho, H. (1978): [Cytogenetical analysis of parthenogenesis of common liver flukes in Japan.] Proc. 31th Kyushu Reg. Meeting Zool. Soc. Jap. (In Japanese)
8) Kitajima, E. W., Paraense, W. L. and Correa, L. R. (1976): The fine structure of *Schistosoma mansoni* sperm (Trematoda: Digenea). J. Parasit., 62, 215–221.
9) Miyazaki, I. (1977): [A newly introduced question on *Paragonimus westermani* (Kerbert, 1878).] Jap. Med. J., (2788), 43–48. (In Japanese)
10) Miyazaki, I. (1978a): [Two types of the lung fluke which has been called *Paragonimus westermani* (Kerbert, 1878).] Jap. Med J., (2819), 43–48. (In Japanese)
11) Miyazaki, I. (1978b): Two types of the lung fluke which has been called *Paragonimus westermani* (Kerbert, 1878). Med. Bull. Fukuoka Univ., 5, 251–263.
12) Moriyama, N. and Terasaki, K. (1979): Spermatogenesis in the two types (diploid type and triploid type) of Japanese common liver fluke, *Fasciola* sp. Jap. J. Parasit., 28 (Suppl.), 29. (In Japanese)
13) Moriyama, N. and Tsuji, M. (1977): Chromosomal and Phenotypical variations in Japanese *Fasciola* sp. Jap. J. Parasit., 26 (Suppl.), 54. (In Japanese)

14) Moriyama, N., Tsuji, M. and Seto, T. (1979): Three karyotypes and their phenotypes of Japanese liver flukes (*Fasciola* sp.). Jap. J. Parasit., 28, 23-33. (In Japanese with English summary)
15) Nez, M. M. and Short, R. B. (1957): Gametogenesis in *Schistosomatium douthitti* (Cort) (Schistosomatidae: Trematoda). J. Parasit., 43, 167-182.
16) Otsuji, Y., Maeda, T., Harada, R., Nakashima, A., Yong, P. K. and Irie, Y. (1976): The ultra-structure of the germ cells of helminths. Med. J. Kagoshima Univ., 28, 933-974. (In Japanese with English summary)
17) Sakaguchi, Y., Kusano, M. and Kihara, S. (1979): Studies on the chromosome of helminths. (11) Chromosomes of *Fasciola* sp. obtained from cattle in Korea. Jap. J. Parasit., 28 (Suppl.), 29. (In Japanese)
18) Sakaguchi, Y. and Tada, I. (1976): Chromosomes of a lung fluke, *Paragonimus westermani*. Chromosome Inf. Serv., (20), 23-24.
19) Sato, M., Oh, M. and Sakoda, K, (1976): Electron microscopic study of spermatogenesis in the lung fluke (*Paragonimus miyazakii*). Z. Zellforsch., 77, 232-243.
20) Terasaki, K. (1977): Studies on Chromosomes of the lung fluke in Japan. Jap. J. Parasit., 26, 222-229.
21) Terasaki. K. (1978): [Chromosomes of original type of *Paragonimus westermani*.] Proc. 31th Kyushu Reg. Meeting Zool. Soc. Jap. (In Japanese)
22) Terasaki. K. (1979): The karyotype of *Paragonimus pulmonalis* collected in Korea. Tenth Seminer on Tropical Medicine to be held in Seoul Korea.

## ウエステルマン肺吸虫およびベルツ肺吸虫の生殖細胞の組織学的観察

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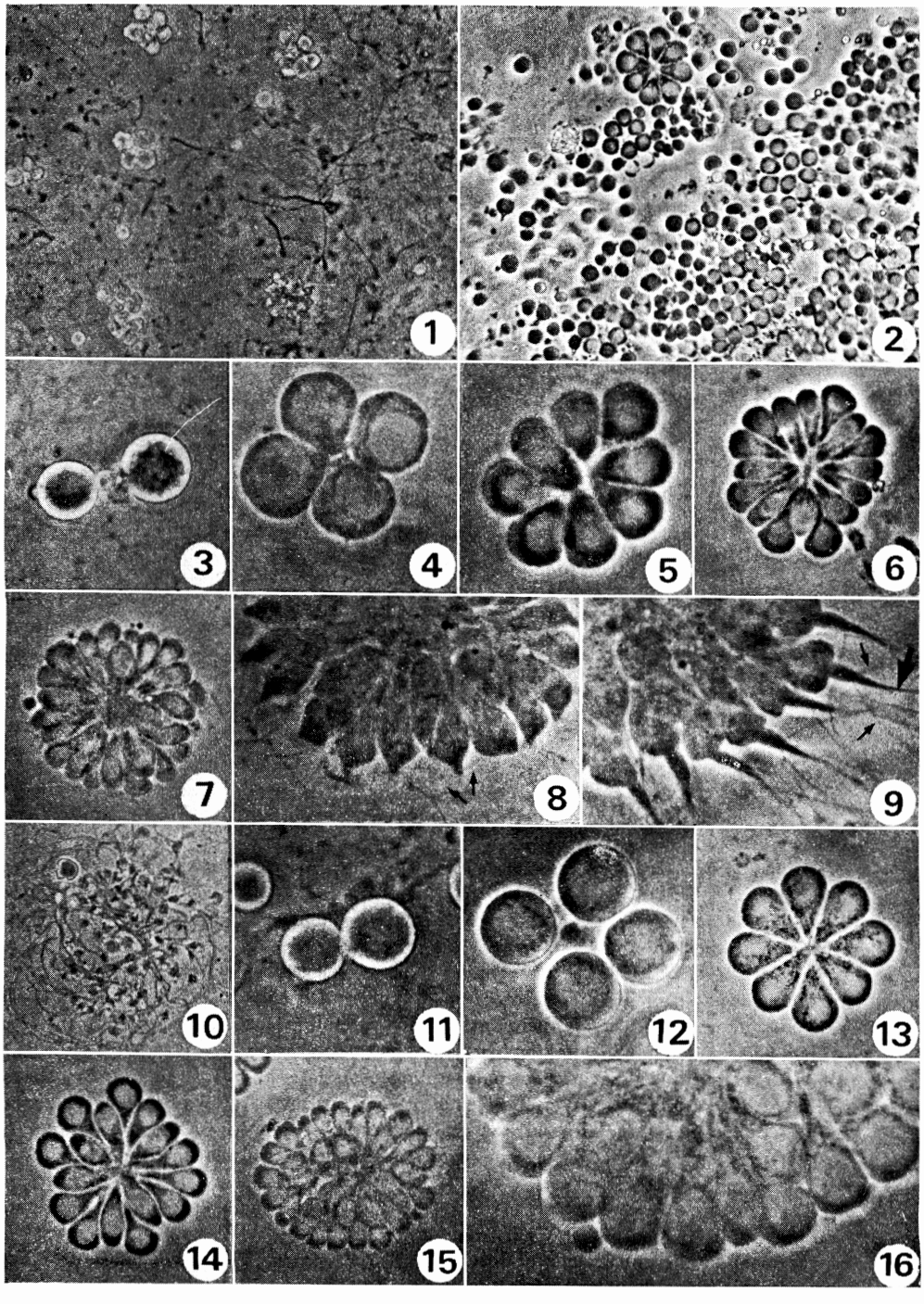
ベルツ肺吸虫は配偶子形成に異常があることが知られているが，その精子形成についての十分な形態学的報告はなされていない．そこで配偶子形成に異常を認めないウエステルマン肺吸虫と比較しながら両種の精巣，卵巣および受精嚢内の生殖細胞を位相差顕微鏡で観察した．また，エアードライ法で作られた精巣および卵巣も合わせて観察した．その結果，ウェステルマン肺吸虫の精子形成過程は，これまで知られている他の肺吸虫（大平肺吸虫と宮崎肺吸虫）とよく類似していたが，ベルツ肺吸虫では，ほとんどの生殖細胞は32-細胞期(初期精子細胞)までで止まり，それ以後精子変態に必要な核濃縮が行なわれていないことがわかつた．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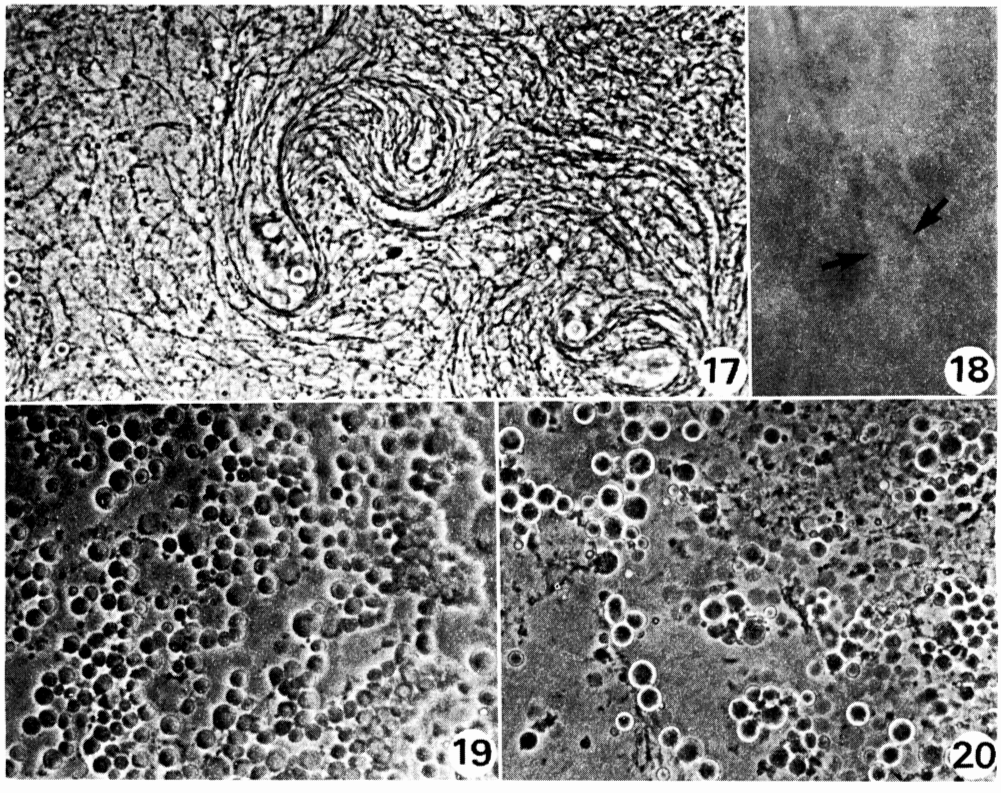



**Explanation of Figures**

Phase-contrast micrographs (Figs. 1, 3–10, 17–19 are *P. westermani* and 2, 11–16, 20 are *P. pulmonalis*)

Fig. 1 Testis including spermatogonia, spermatocytes and spermatozoa in various stages of spermatogenesis.

Fig. 2 Testis including permatogonia and spermatocytes without spermatozoon in various stages of spermatogenesis.

Figs. 3–7 Each rosette of 2 and 4 spermatogonia, 8 and 16 spermatocytes, and 32 spermatids.

Fig. 8 Spermatogenesis showing rosette of 32 spermatids which have just begun to differentiate into spermatozoa by forming pairs of flagella extending in a lateral direction. Note a pair of flagella extending laterally from each spermatid (arrows).

Fig. 9 A rosette of spermatids at a stage of spermiogenesis later than that in Fig. 8. Note the region of differentiation at the apical ends of each spermatid. Two flagella (small arrows) and a cellular projection (large arrow) grow out from spermatid.

Fig. 10 A rosette of spermatids at a stage of spermiogenesis later than that in Fig. 9. Spermatids are linked with the cell bodies.

Figs. 11–15 Each rosette of 2 and 4 spermatogonia, 8 and 16 spermatocytes, and 32 spermatids. They resemble to those of *P. westermani* (Figs. 3–7).

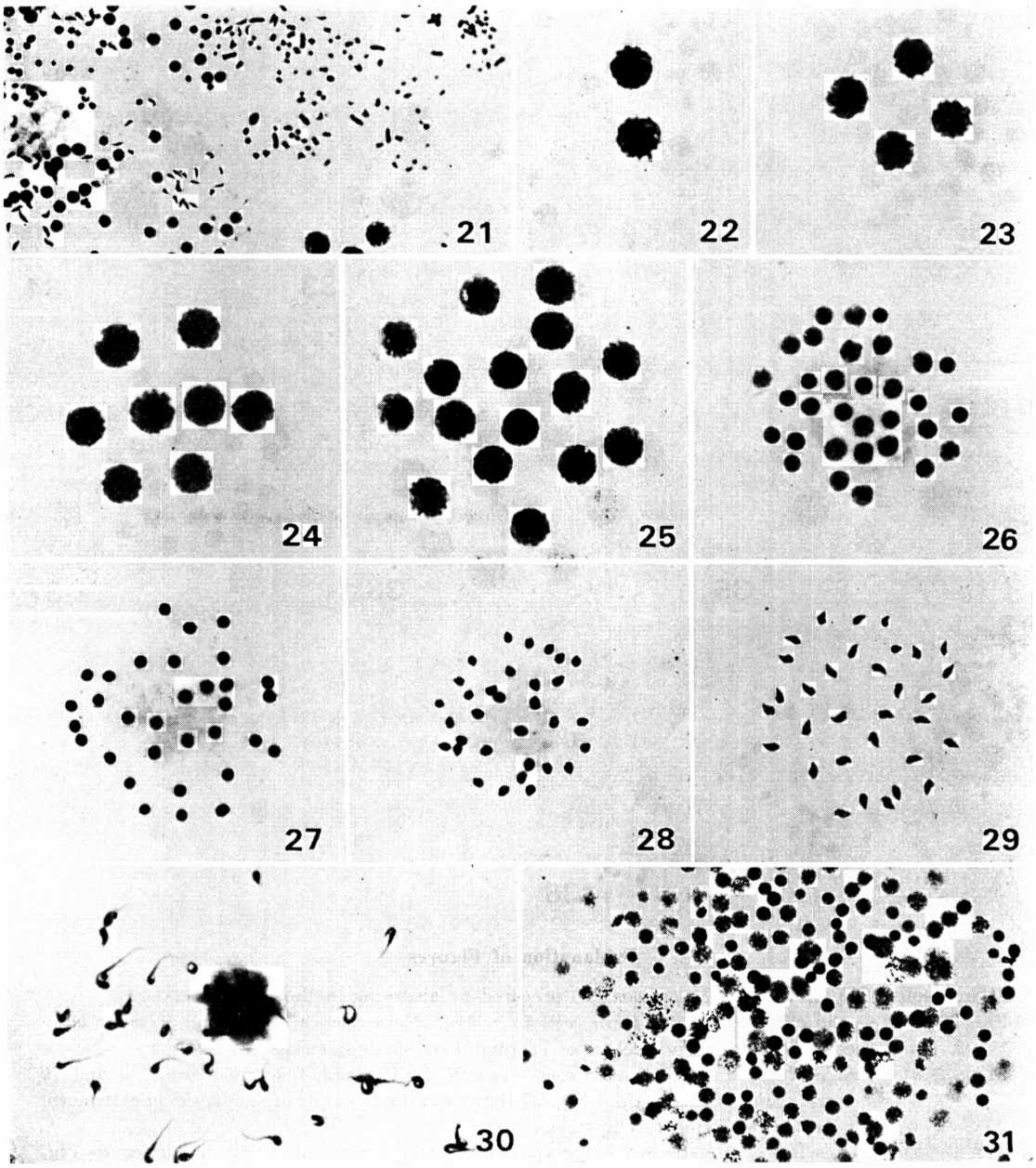
Fig. 16 Enlargement of the apical ends of rosette of 32 spermatids. Note the apical ends showing a smooth tip without any projection.

Fig. 17 Numerous spermatozoa in seminal receptacle.

Fig. 18 Bifurcated end of a spermatozoon.

Fig. 19 Ovarian cells of *P. westermani*.

Fig. 20 Ovarian cells of *P. pulmonalis* similar to Fig. 19.




**Explanation of Figures**

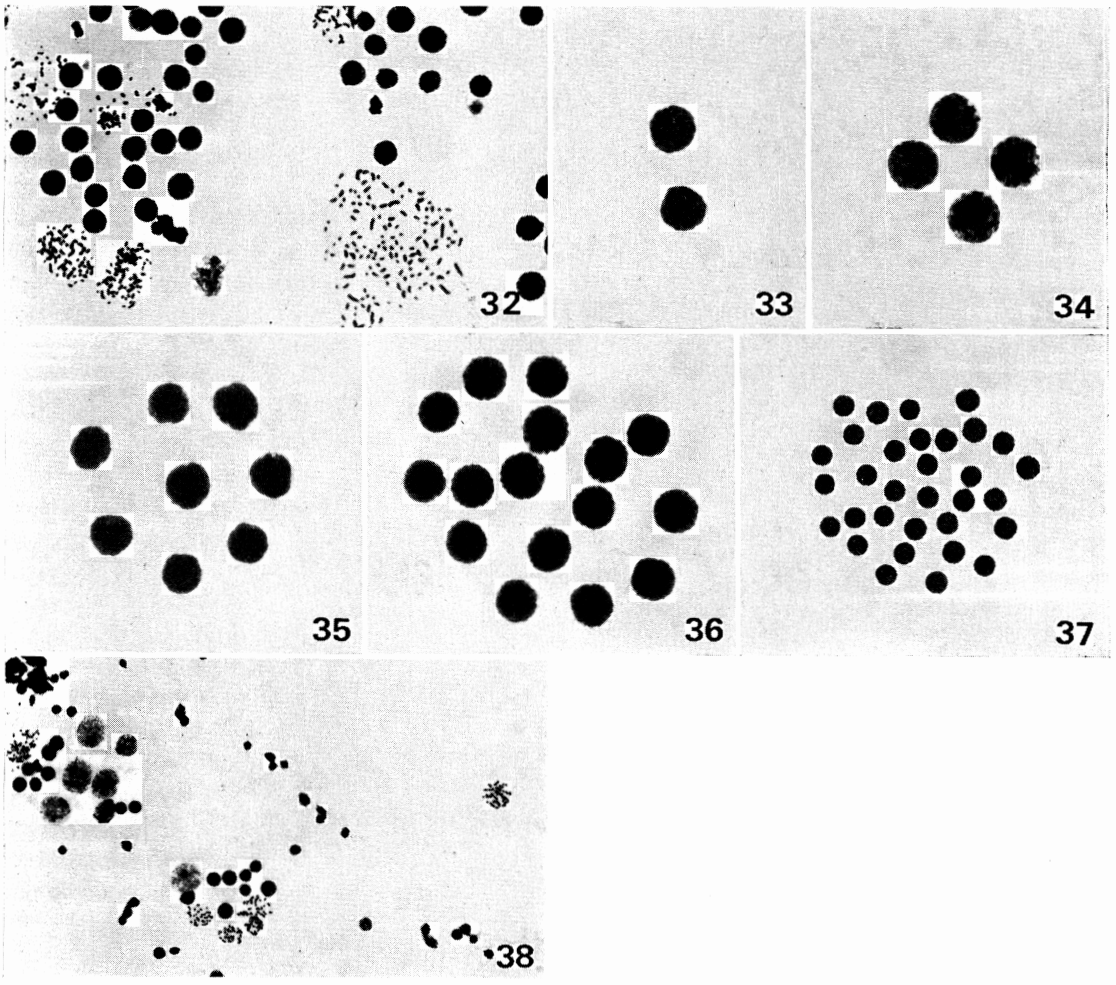
Micrographs of specimens (of *P. westermani*) prepared by airdrying method.

Fig. 21 Nuclei of germ cells in testis showing in various stages of spermatogenesis. Note masses of chromosomes and spermatozoa in various stages of spermiogenesis.

Figs. 22-27 Each nucleus corresponding to each "rosette" of 2 and 4 spermatogonia, 8 and 16 spermatocytes, and 32 spermatid. Fig. 26 shows swelling of cells of spermatid in early stage and Fig. 27 shows condensation of chromatin of spermatid.

Figs. 28-30 Nuclei of spermatid in various stages of spermiogenesis.

Fig. 31 Ovary showing germ cells and karyotype plates to be disjointed.




**Explanation of Figures**

Micrographs of specimens (of *P. pulmonalis*) prepared by airdrying method.

Fig. 32 Nuclei and chromosomes of germ cells in testis. Note masses consisting of many chromosomes and without any nucleus of spermatid on spermiogenesis.

Figs. 33–37 Each nucleus corresponding to each "rosette" of 2 and 4 spermatogonia, 8 and 16 spermatocyte, and 32 spermatid. Fig. 37 shows swelling of cells of spermatid in early stage without any condensation of chromatin.

Fig. 38 Ovary showing germ cells and karyotype plates to be disjointed. This resembles to Fig. 31 except the number of chromosome and the absense of meiosis.