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The Central Nervous System of Heterosymelic Individuals of the Spider *Tegenaria atrica*

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This paper presents the results of research on the nervous system in individuals of *Tegenaria atrica* with heterosymely of walking legs, a pedipalp and the first walking leg. Temperatures alternating between 14° and 32°C every 12 hours were applied as a teratogenic factor in the early stage of embryogenesis. From the thus obtained specimens, histological sections were taken using the paraffin method and then stained. Analysis of the nervous system in individuals with partial heterosymely of walking legs showed no significant changes in the structure of subesophageal nervous mass. In most of the studied individuals with partial and total heterosymely of a pedipalp and walking leg no changes were observed in the relevant parts of the nervous system either, although in two cases a fusion of nerve ganglia was noted, from which the nerves branched off towards the conjoined legs.

Key words: Alternating temperatures, anomaly, Arachnida, central nervous system, teratological studies.

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The central nervous system of spiders has attracted great interest of arachnologists for many years. The extensive work of BABU (1965) is worth mentioning, in which the author discusses in detail the anatomical and histological structure of this system in the spider *Poecilotheria* sp., comparing it with investigated members of other orders of arachnids. Subsequent works by the same author are a continuation and extension of observations by further research aspects in *Poecilotheria* (1969) and *Argiope aurantia* (1975). BABU and BARTH (1984) also analyzed the central nervous system in the spider *Cupiennius salei* and HWANG and MOON (2003) in *Achaearanea tepidariorum*. Despite many publications, the structure of this system is still not thoroughly examined. This particularly applies to the superior unit, which is the brain of arachnids, especially in spiders. There are different views on the segmental structure of this component of the system (BABU & BARTH 1984; DAMEN *et al*. 1998; LEGENDRE 1979; MITTMAN & SCHOLTZ 2003; TELFORD & THOMAS 1998)

The central nervous system of spiders has a high concentration of ganglia and is completely contained in the prosoma (BABU 1965; IVANOV 1965; PUNZO 2007). Two main parts are distinguished in the nervous system: the supraesophageal mass and the subesophageal mass. They are interconnected by short and thick esophageal connectives. The supraesophageal ganglion called the "brain" is situated on the subesophageal ganglion in the anterior part of the prosoma and gathers eye centers and cheliceral and pedipalpal ganglia. The subesophageal ganglion consists mainly of the fused ganglia of the walking legs and the opisthosomal ganglia. True opisthosomal ganglia exist only in embryos and during development they migrate into the prosoma where they fuse with ganglia of the walking appendages, enlarging the subesophageal mass (FOELIX 1996; IVANOV 1965). The ganglia of walking legs are relatively large, separated from each other and together form a broad, star-shaped structure. The arrangement of this structure defines the internal metamerism of the larger part of the prosoma. Both main parts of the central nervous system are characterized by the same histological structure. There is a clear division here into a marginal layer of neurons forming the cortex and central, compact and dense mass of nerve fibers constituting the neuropil (FOELIX 1996; PUNZO 2007). As evidenced by previous studies conducted on the nervous system of *Tegenaria*, the structure of this system, as well as other internal systems, can be seriously disturbed in certain developmental anomalies occurring spontaneously or induced experimentally, such as oligomely or polymely. The consequence of the development of additional legs is the presence of supernumerary nervous ganglia in the nervous mass, and the lack of legs causes depletion of the total volume of this system. The extent of these changes always depends on the number of legs occurring on the prosoma (JACUŃSKI *et al*. 2002b, 2005; NAPIÓRKOWSKA *et al*. 2006). Heterosymely is yet another, experimentally induced, equally interesting and relatively often observed malformation of the spider body. This anomaly consists of fusion of legs and structures of appendage origin, situated next to each other, and thus on the same side of the body. It may be of varying depth and include only the proximal part of the appendages (partial heterosymely) or all of their segments (total heterosymely). In individuals affected by this developmental monstrosity, apart from changes in the external structure of the body, such as lateral dislocation of the fovea media and distortion of the sternum, there may occur significant changes in the construction of the internal organs (JACUŃSKI 1984). In this case, information about changes in the anatomy of these spiders is still insufficient, particularly as regards the structure of the central nervous system. Only MIKULSKA and KOKOCIŃSKI (1966) present a case of spontaneous partial heterosymely of the third and fourth leg in the spider *Agelena labyrinthica* Clerck, in which innervation of this complex was examined. Their histological analysis revealed the presence of two independent leg nerves, located far apart from each other. Therefore, in recent years, research has been undertaken to analyze the structure of the neural mass on a larger group of spiders with heterosymely of walking legs, as well as with heterosymely of a walking leg and pedipalp.

Material and Methods

Experimental studies were conducted on individuals of the spider *Tegenaria atrica* C.L.Koch 1943. Embryos were obtained from females caught in 2005-2010 during the summer months, mostly near the towns of Toruń and Chełmża (Poland), and stored in the laboratory. Each specimen was kept separately in a 250 cm^3 glass vessel under optimal conditions for the species, i.e. a temperature of 21-23^{o}C and relative humidity of ca.70% (MIKULSKA & JACUŃSKI 1968; JACUŃSKI *et al*. 1994). Each glass vessel was well ventilated and equipped with a cotton ball soaked with water. After a short period of adjustment to laboratory conditions, each sexually mature female was kept together with a male for a few hours for complete fertilization. The first cocoons were laid after a few weeks, and each successive deposit containing developing embryos was counted and divided into two parts. One part constituted a control sample and was kept in conditions optimal for embryonic development (23^{o}C, humidity 70%) (JACUŃSKI 1970; JACUŃSKI & WIŚNIEWSKI 1997), whereas the other one was exposed to the teratogenic factor. In this experiment, the temperatures 14^{o}C and 32^{o}C, applied alternately every 12 hours for 10 days constituted the teratogenic factor. The temperatures were applied until the first metameres of the prosoma appeared on the germ band. As in the case of the control sample, further incubation of the embryos was continued at the temperature optimal for embryonic development. Once the larvae hatched, each of them was carefully examined for developmental anomalies. Individuals with heterosymely of walking legs and heterosymely of a walking leg and pedipalp were reared in separate dishes until the stage of nymph II, and then they were fixed in Bouin's fluid. Histological sections were made using the paraffin method, and then they were stained with Meyer's hematoxylin and eosin according to ZAWISTOWSKI (1986). The particular developmental stages of this spider species were defined following the nomenclature of VACHON (1957).

Results

Teratological experiments were conducted on ca. 10,000 embryos during six breeding seasons. Half of the total number of embryos constituted the control sample in which no developmental abnormalities were found, whereas in the other half exposed to alternate effects of two temperatures, 674 specimens were found with all types of malformation of appendages and structures of appendage origin so far confirmed in *Tegenaria*. The mortality rate of embryos was quite high, about 20%, while in the control it was at a level of about 4%. Among the individuals with anomalies, the largest group consisted of specimens with unilateral or bilateral oligomely of legs (i.e. reduction in the number of legs). They constituted 67.21% (453 individuals) of all specimens with anomalies. Schistomely, i.e. bifurcated legs occurred in 9.05% of individuals (61 larvae), while larvae with a shortened appendage made up 6.68% (45 individuals). Polymely (supernumerary legs) occurred in 3.41% of individuals (23 larvae), while symely (fusion of

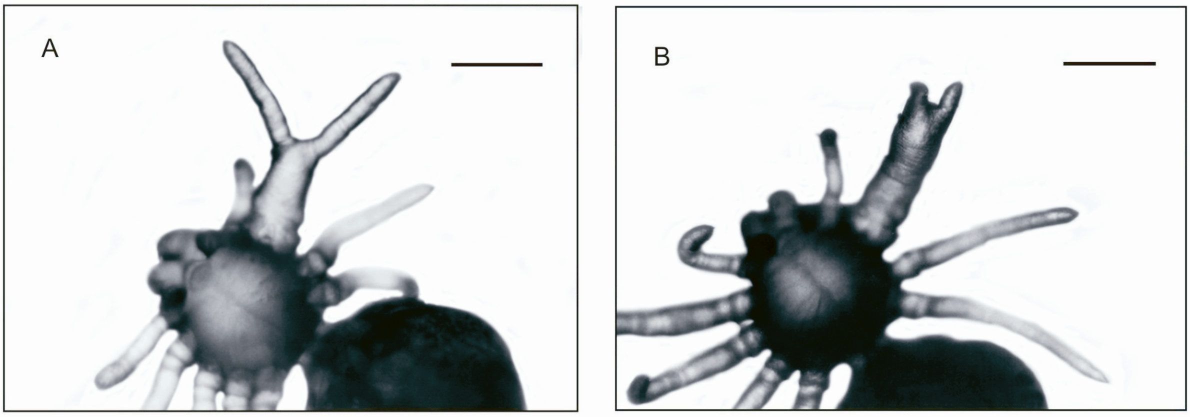

Fig. 1A-B. *Tegenaria atrica* larva with heterosymely of the first and the second walking legs (ventral view): A – with partial heterosymely, scale bar = 0.47 mm; B – with deep heterosymely, scale bar = 0.44 mm.

legs located on the opposite side of the prosoma) in only 2.23% of individuals (15 larvae). Larvae with complex anomalies and the so-called other deformities occurred sporadically: 1.34% (9 individuals) and 0.59% (4 individuals), respectively.

In the teratological material, there were 64 larvae with heterosymely, and thus with fusion of legs situated next to each other, i.e. just 9.50% of specimens affected by malformations. Fusion of walking legs was found in 39 specimens, while fusion of the first walking leg and pedipalp was observed in 25 specimens. It was observed that in most cases fusions occurred on the right side of the body (70.31% – 45 individuals). The walking legs were never fused along their entire length. In 12 cases of this monstrosity, coxae, trochanters and femurs of walking legs were fused, in 11 cases – only coxae and trochanters, and in 10 individuals the fusion was more serious and included segments from the coxae to patella (Fig. 1A). Heterosymely of six segments of walking legs, with only the last segments (tarsus) free, was found only in six individuals (Fig. 1B). At the same time, the teratogenic factor led to fusion of the first and the second leg (22 individuals), and the second and the third leg (17 individuals), whereas there was no fusion of the third and the fourth leg.

Analysis of a series of histological sections showed no significant changes in the construction and arrangement of individual ganglia in the subesophageal nervous mass. The size of ganglia of fused appendages (Fig. 2A) did not differ from the size of ganglia of walking legs of control individuals. They were of normal size, clearly defined and separated from each other (Fig. 2B). From each ganglion the nerves ran in the direction of the relevant part of the heterosymelic complex (Fig. 2C). In the large common part of the complex, two nerves were always located side by side (Fig. 2D), and in the free ends of legs, the nerves were centrally located (Fig. 2E).

Heterosymely of a pedipalp, which has six segments, and the first walking leg, which has seven segments, occurred in 25 specimens, including 19 specimens with partial and 6 specimens with total heterosymely. Partial heterosymely often involved the first two segments of legs (8 cases), in 7 larvae the fusion of femur segments occurred, and in 4 larvae – fusion of the patella. In total heterosymely, all segments of legs were fused together along the entire length, and an arched bend toward the mouth was a characteristic feature of these legs.

Analysis of the structure of the central nervous system on horizontal and sagittal sections of individuals with partial heterosymely revealed that ganglia of legs were separated from each other and well visible (Fig. 3A, B). Separate nerves ran from these ganglia towards the fused legs. Only in one case (Fig. 3C, D), a ganglion of a pedipalp was fused with a ganglion of a walking leg. The total heterosymely of a pedipalp and a walking leg was less frequent, and therefore histological analysis was possible for four specimens. Fusion of ganglia of these legs was well visible on a horizontal cross-section (Fig. 3E, F) only in one case.

Discussion

Anomalies occurring in the prosoma lead to changes in the internal structure of such individuals, in which malformations in the structure of the central nervous system can be expected inter alia. So far the nervous system has been examined in oligomelic (JACUŃSKI *et al*. 2005) and in polymelic individuals (JACUŃSKI *et al*. 2002b; NAPIÓRKOWSKA *et al*. 2006). However, in this respect,

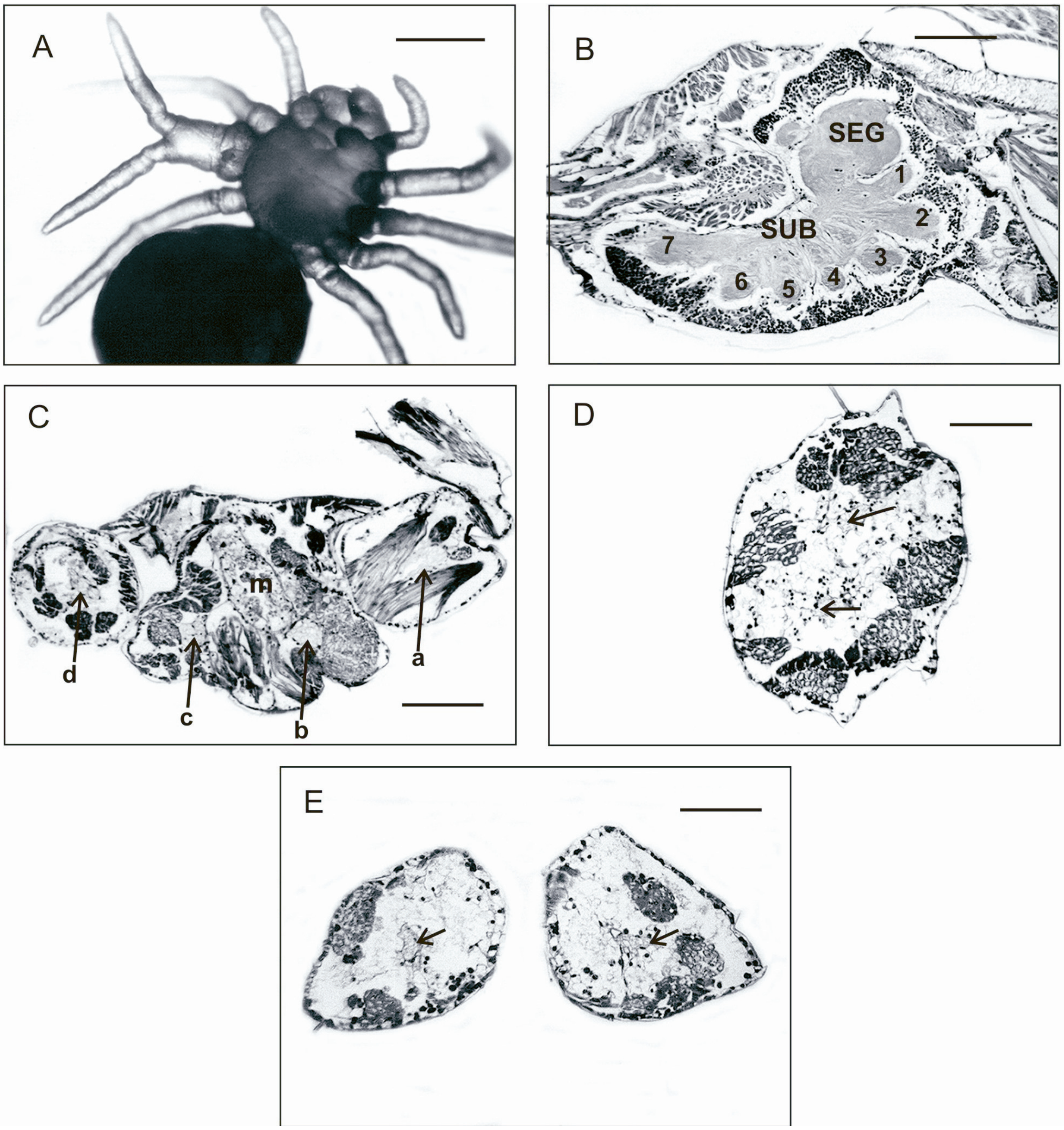

Fig. 2A-E. A. *Tegenaria atrica* larva with partial heterosymely of the second and the third walking legs (ventral view), scale bar = 0.38 mm; B – parasagittal section through the prosoma and the central nervous system: SEG – supraesophageal ganglion, SUB – subesophageal ganglion, 1 – cheliceral ganglia, 2 – pedipalp ganglia, 3-6 walking legs ganglia, 7 – abdomen ganglia, scale bar = 0.20 mm; C – parasagittal section along the edge of cephalothorax: a – cross section of the first leg and its nerve, b, c – cross section through a common part of the second and third leg and their nerves, d – cross section through the fourth leg and its nerve, m – fused midgut diverticula, scale bar = 0.20 mm; D –section through a common part of the heterosymelic complex, arrows indicate nerves, scale bar = 0.14 mm; E – cross section through two free ends of heterosymelic legs, arrows indicate the location of the nerves, scale bar = 0.10 mm.

heterosymely is the least known deformation of the prosoma. The structure of the central nervous system had been determined in only one individual with partial heterosymely of walking legs (MIKULSKA & KOKOCIŃSKI 1966) and in only one individual with total heterosymely of chelicera and pedipalp (JACUŃSKI *et al*. 2002a). In both cases, no significant disturbances in neuromery were observed. Despite the fusion of legs, there was no fusion of relevant ganglia. Therefore, studies were undertaken in recent years to investigate the nervous system in a larger group of heterosymelic individuals to determine whether the nervous system always retains its correct structure in the case of the fusion of legs.

In six breeding seasons, it was found in only 9.50 % of all individuals affected by this deformation of the prosoma. In morphological terms, heterosymely resembles schistomely (bifurcation of legs), while in heterosymely observed in spiders, two appendages located next to each other are fused. The common feature of these anomalies is a very small mobility of the modified appendages, while their thickness is the basic difference that enables the

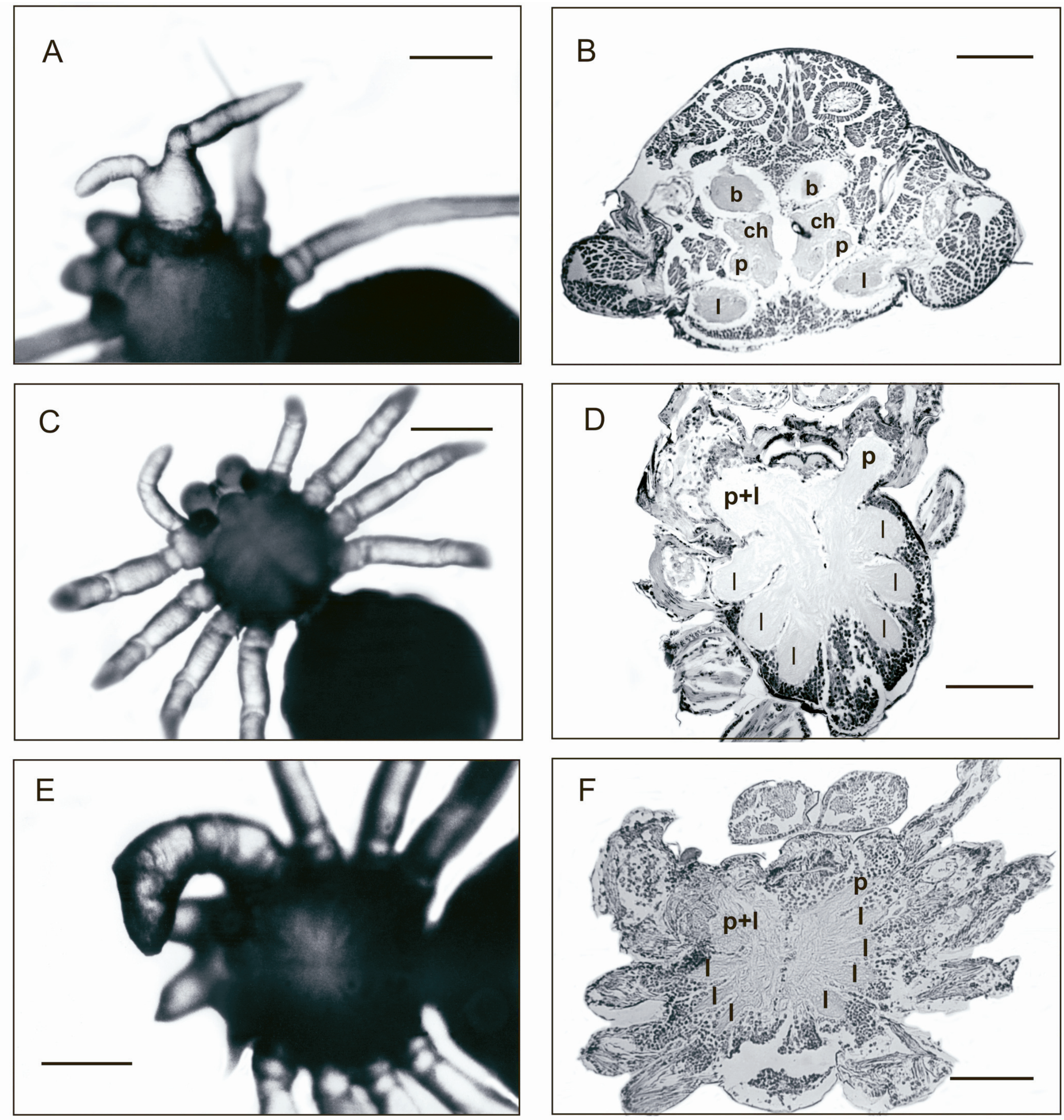

Fig. 3A-F. *Tegenaria atrica* larva with heterosymely of a pedipalp and walking leg (ventral view) and their cross sections through the prosoma: A – larva with partial heterosymely of legs, scale bar = 0.34 mm; B – section through the cephalic part of prosoma: b – brain, ch – cheliceral ganglia, l – ganglia of walking legs, scale bar = 0.21 mm; C – larva with shallow heterosymely of legs (ventral view), scale bar = 0.40 mm; D – the horizontal section: p – pedipalpal ganglion, p+l – ganglia of a pedipalp and a leg, l – ganglia of walking legs, scale bar = 0.21 mm; E – larva with total heterosymely (ventral view), scale bar = 0.26 mm; F – the horizontal section: p+l – ganglia of a pedipalp and a walking leg, scale bar = 0.18 mm.

classification of these two deformations. A heterosymelic complex is typically two times thicker than the legs in the control sample which was not observed in the case of schistomely. NAPIÓRKOWSKA *et al*. (2007, 2009-2010), who studied the possibilities of regeneration and spontaneous repair processes of modified appendages, found that both schistomelic and heterosymelic legs are subject to epimorphosis, but repair processes occur only in the case of schistomely. Indeed after each molt, the length of the free ends is gradually reduced until their complete disappearance. The repair processes did not occur in the previously studied cases of heterosymely and the depth of anomalies remained unchanged. The most important and explicit feature, enabling these anomalies to be distinguished from each other, is the structure of the nervous system. Preliminary research on the nervous system of schistomelic specimens indicates (unpublished data) that the structure of the central nervous system does not change. The ganglion of the schistomelic leg is well developed and does not differ in size from other leg ganglia. Bifurcation of the leg nerve is observed only at the place where the appendage bifurcates. The analysis of histological sections of specimens with partial heterosymely of walking legs shows that in the subesophageal nervous mass, ganglia of legs were always well developed and separated from each other, with nerves running in the direction of the appropriate appendages. The fusion of legs does not disturb the course of these nerves, which are always situated next to each other in the fused parts of the legs. However, the nervous system in the total heterosymely of legs was not examined, which seems to be an extremely important aspect of the research. However, other and ambiguous conclusions can be drawn from analysis of the nervous system in the case of heterosymely of a pedipalp and a walking leg. Most of the cases of partial heterosymely show that there are separate ganglia for a pedipalp and a walking leg, from which separate nerves run. However, the situation was different in one case, namely, there was a fusion of a pedipalp ganglion with a ganglion of a walking leg. Similarly one large ganglion was observed only in one case with total heterosymely of a pedipalp and a walking leg. This ganglion developed from the fusion of ganglia of these two legs, different in terms of structure and function. It can be concluded that the nervous system usually retains its proper construction. Heterosymely, however, is an exception to this rule. Heterosymely is an anomaly in which disturbances in the structure of the digestive system also occur. JACUŃSKI (1983) found that fusion of legs may be followed by fusion of the corresponding midgut diverticula.

The direct causes of heterosymely are unknown. Biochemical processes are responsible for the occurrence of this monstrosity during embryogenesis. Such studies are very difficult at this stage for methodological reasons, and at present this phenomenon is analyzed by studying the defects that become apparent during the development of embryos treated with alternating temperatures. It has been found (JACUŃSKI 1984; JACUŃSKI 2002) that thermal shocks affect changes in the formation of blastoderm. These changes are caused by faulty or delayed migration of descendant nuclei on the yolk surface, incorrect cell movement and faulty localization of blastomeres and finally cell death. Gaps in the form of cavities or cracks develop in the blastoderm, in which the yolk appears with a modified, more liquid consistency. Gaps in the blastoderm eliminate certain parts of the embryo, which is a probable cause of oligomely, while approaching fragments of the blastoderm, usually situated far apart, can lead to heterosymely – the anomaly discussed in this paper.

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