PART III

HYPOTHESES ABOUT THE PATHOPHYSIOLOGY OF NON-COMMUNICABLE DISEASES

. . . .we can gain experience without making experiments, solely by reasoning appropriately about well-established facts, just as we can make experiments and observations without gaining experience, if we limit ourselves to noting facts.

— Claude Bernard (1813–1878)

INTRODUCTION

> Without theory, practice is but routine born of habit. Theory alone can bring forth and develop the spirit of invention.
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> . . .
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> A theoretical discovery has but the merits of its existence. It awakens hope, and that is all. But let it be cultivated, let it grow, and you will see what it will become.
>
> Louis Pasteur, to his pupils at Lille

Taeniid and *Toxocara* larvae that live in humans as accidental hosts live a juvenile life without prospects to enter the adult reproductive stage in the intestine of a definitive host. Humans are the sole exception from the general rule that all animals are usually eaten by predators. The larvae are probably unaware of their tragic destiny, and they presumably live their life as if they were actually living in a prey animal with the prospect of being eaten by their definitive host. No matter the vitality of the larvae when the human host dies, the life of the larvae will be confined to the accidental host. The impact that the larvae might have on their human host is what I explore in this part of the book.

So far the focus on different taeniid larval tissue infections (LTI) that are transmitted from a non-human definitive host has mostly been related to the mechanical impact of the macroscopically visible cyst. In this part of the book, I present several reasons to expand this view. To acknowledge the capacities of helminths, I first account for some aspects of helminth biology. I then suggest a model of the pathophysiology of taeniid and *Toxocara* LTI that reflects the different stages of the life and death of the larvae. These stages are to some extent common to both taeniid and *Toxocara*. Furthermore, I suggest that the aetiology of certain human diseases is related to different stages of taeniid and *Toxocara* LTI.

The *Toxocara* larva is unable to grow in an intermediate host. Therefore, a single taeniid larva has a greater pathogenic potential than a single *Toxocara* larva because of the taeniid's ability to grow and increase its total biomass in the intermediate host. Some taeniid larvae are even able to reproduce asexually, and in this way, the metacestode may exhibit a very long life expectancy. A taeniid metacestode may therefore affect the host as long as the host stays alive, whereas the *Toxocara* larva has a life expectancy of approximately 10 years.

Furthermore, taeniid eggs are infectious from the moment they leave the dog with the faeces, whereas *Toxocara* eggs need to mature in the soil for a few days after

leaving the dog. For this reason, dog ownership poses a greater risk of transmission of infectious taeniid eggs than infectious *Toxocara* eggs to the human family members. On the other hand, the prevalence of *Toxocara* in dogs and cats is probably higher than the prevalence of taeniids.

According to the literature, *Toxocara* LTI is much more susceptible to albendazole treatment than taeniid LTI, perhaps with the exception of *T. solium* LTI. The symptoms alleviated by albendazole treatment recurred during treatment discontinuation in the patients described in Part I, indicating that taeniid LTI is a more important cause of disease in humans than *Toxocara* LTI.

In any case, both taeniid and *Toxocara* LTI theoretically cause similar symptoms, as there are many biological similarities between these types of infection. Nevertheless, *Taenia* LTI transmitted from a non-human definitive host is probably by far the most important helminth pathogen in humans in wealthy and well-organized societies today.

BASIC HELMINTH BIOLOGY

> But one major difference between predator-prey and parasite-host systems is that in the former the actors are usually visible to each other, with scenes happening before your eyes even when played out at such vastly different scales as a lion capturing a buffalo or a spider trapping a fly. In parasite-host systems one of the protagonists is, at best, difficult to observe. (1)
>
> Claude Combes (1935–)

When considering possible pathophysiological mechanisms for taeniid and *Toxocara* LTI, it is important to remember that helminths and humans are both multicellular organisms and members of the kingdom of Animalia or Metazoa. The size of adult cestodes compared with adult humans differs much more than the difference between David and Goliath. The size of the juvenile stage of the taeniid and *Toxocara* larvae compared with human children differs even more. However, despite the great differences in size, the fact that all multicellular organisms share many fundamental physiological functions should not be ignored.

Cell signalling

The most important physiological difference between unicellular and multicellular organisms is the fundamental need for communication between the cells comprising the multicellular organism. (2) Communication between cells is mediated mainly by extracellular signal molecules and, like any other type of effective communication, it takes place mainly as a dialogue. To accomplish the dialogue, most cells both emit and receive signals. Animals use a large number of signal proteins, receptors, and intracellular signalling proteins. The structure and design within proteins during the evolution of animals have been remarkably conserved, and both worms and mammals use essentially similar machinery for cell communication. (2)

1. Combes C. Parasitism: the ecology and evolution of intimate interactions. The University of Chicago Press, 2001; p. 581.

2. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 5th ed. Garland Science Taylor and Francis Group, 2008.

Primitive multicellular helminth-like organisms appeared around 800 million years ago, the divergence of vertebrates from the other animal groups occurred around 600 million years ago, and mammals arrived on the scene around 300 million years ago. (3) About 3000 free-living and 10 000 parasitic flatworm species have been identified, and they occupy every possible niche in their vertebrate hosts. (4) Hence, the relationships and interactions between parasitic helminths and our evolutionary vertebrate ancestors as well as the earliest hominids have lasted for millions of years and the potential for evolution of sophisticated interactions between flatworms and vertebrate hosts has been immense.

The multicellular nature of individual organisms paved the way for functional delegation such that single cells or groups of cells could become specialized in various ways for feeding, reproduction, and mobility, as well as for the integration of all of these functions. (5) To accomplish communication between these specialized cells, a great number of extracellular cell signalling molecules are in use. These signalling molecules may roughly be divided into groups of hormones, cytokines, growth factors, and neurotransmitters and act together like a huge orchestra where hundreds of instruments are played at the same time. (6,7) The perception of the various signalling molecules differs among different cells, and the intracellular cascade of events elicited by identical signalling molecules may differ among different cells as well. Cell signalling is thus a complex web of events.

Extracellular cell signalling molecules and receptors and intracellular signalling cascades in flatworms are still a relatively unexplored area, encompassing only a limited number of parasitic flatworms such as *Schistosoma*, *Fasciola*, *Hymenolepis*, and *Echinococcus*. However, various components comprising the classic pathways known in mammals can be found among the known flatworm signalling proteins. (8) The implication of signal transduction pathways shared by humans and flatworms is the possibility of "crosstalk" between the helminths and the host by a partly "common language." Dysfunctional cell signalling is responsible for a plethora of diseases and conditions, (6) and helminths possess the ability to cause dysfunctions of cell signalling in the host.

The helminth nervous system and neurotransmitters

The nerve cell arose early in metazoan evolution to meet the need for integration of groups of specialized cells. Neuropeptides are signalling molecules in all metazoans possessing a nervous system (such as cestodes and humans), and

3. Walker RJ, Brooks HL, Holden-Dye L. Evolution and overview of classical transmitter molecules and their receptors. *Parasitology* 1996;113:S3-S33.

4. Gustafsson MKS, Halton DW, Kreshchenko ND, Movsessian SO, Raikova OI, Reuter M, Terenina NB. Neuropeptides in flatworms [Review]. *Peptides* 2002;23:2053-61.

5. Shaw C. Neuropeptides and their evolution. *Parasitology* 1996;113:S35-S45.

6. Hancock JT. Cell signalling. 3rd ed. Oxford University Press, 2010; p. 56.

7. Hancock JT. Cell signalling is the music of life. *Br J Biomed Sci* 2008; 65:205-8.

8. Yoshino TP, Vermeire JJ, Humphries JE. Signal transduction at the host-parasite interface. In: Maule AG, Marks NJ, eds. Parasitic flatworms: molecular biology, biochemistry, immunology and physiology. CAB International, 2005; pp. 210-27.

6. Hancock JT. Cell signalling. 3rd ed. Oxford University Press, 2010; p. 330.

phylogenetic studies have revealed that peptidic neurotransmission is of early evolutionary origin. (5)

In this presentation of the cestode nervous system, I heavily rely on the impressive work in parasitological research done by Halton, Gustafsson, Shaw, and others. The morphological features of the cestode nervous system consist of a central bilobed brain and longitudinal nerve cords connected by commissures, together with a peripheral array of sensory and motor nerve plexuses. (9) Development of bilateral symmetry most likely necessitated the evolution of the brain, thereby preventing the two sides of the ancestral tapeworm from engaging in contradictory activities. (10) The ganglionic structures of the two brain lobes are connected by a largely fibrous ring-like commissure that originates in the ganglia.

The plan of the flatworm nervous system is the so-called orthogon, a rectilinear, ladder-like configuration of longitudinal nerve cords connected at intervals by transverse ring commissures. (9) The central nervous system (CNS) comprises the bilobed brain and paired longitudinal main nerve cords. The peripheral nervous system (PNS) of cestodes comprises the neurons associated with the main nerve cords and the subepidermal, submuscular, and genital nerve plexuses. The well-developed nerve nets or plexuses dominate the PNS of cestodes. The CNS dominates the nervous system in parasitic flatworms such as cestodes. (9)

The flatworm neuron is that of a secretory cell engaged in the synthesis and export of material by axonal transport in vesicles; both the size and the structure of vesicles are diverse. (9) Chemical signals in flatworms seem to be transmitted as neurotransmitters or neuromodulators, either directly to their targets at synaptic and non-synaptic release sites, or as local chemical mediators that are delivered via the extracellular matrix. (9) Sensory nerves and free nerve terminals penetrate the epithelium or tegument of flatworms, but the evidence for the functional differentiation of sensory endings is meagre. (9)

The seemingly primitive anatomical construction of the platyhelminth nervous system should not be equated with simplicity in function. Adult and developmental stages of parasitic flatworms rely on well-developed neuromuscular systems to coordinate and control a diverse repertoire of physiological and behavioural activities. Their nervous system is based on multifunctional neurons which synthesize and secrete a multitude of neuroactive substances and which resemble the ancestors of the more specialized neurons of higher organisms. (9)

Neuroactive substances of flatworms include acetylcholine, serotonin or 5-hydroxytryptamine (5-HT), the catecholamines noradrenaline and dopamine, histamine, glutamate, γ-amino butyric acid (GABA), and a number of neuropeptides. The biosynthesis of biologically active peptides is an evolutionary old and well-conserved process, and neuropeptides are believed to be the "first" transmitters to have evolved.

5. Shaw C. Neuropeptides and their evolution. *Parasitology* 1996;113:S35-S45.

9. Halton DW, Gustafsson MKS. Functional morphology of the platyhelminth nervous system. *Parasitology* 1996;113:S47-S72.

10. Koopowitz H, Keenan L. The primitive brains of Platyhelminthes. *Trends Neurosci* 1982;3:77-9.

(11,4) The complexity of the peptidic "vocabulary" of invertebrate neurons is of a similar order of magnitude as in vertebrates. (5) Homologues to at least 26 mammalian and six invertebrate peptides have been identified so far. (9) The amino acid sequences of six native flatworm neuropeptides have been determined. (4) These neuropeptides comprise two families:

a. FMRFamide-related peptides
b. Neuropeptide F family

The latter family appears to be the invertebrate homologue to neuropeptide Y, which is exclusively expressed within the nervous system of vertebrates (4) and is released by many sympathetic nerve endings in humans. Neuropeptide Y binds to a specific receptor on cholinergic terminals with an adjuvant inhibitory effect on acetylcholine release. (12)

Neuropeptide immunoreactivities (IRs) using antisera that have been raised largely against vertebrate neuropeptides have been described in the nervous system of flatworms. Substance P immunoreactivity (SP-IR) has been described from the larval and adult cestode *Diphyllobothrium dendriticum*. SP-IR occurs in a separate set of bipolar neurons with long processes extending to the surface. (13)

Serotonin or 5-HT appears to be the dominant biogenic amine in all flatworm species examined and has been shown to induce motility in muscle preparations of the cestode *Hymenolepis diminuta*. (9) A major source of 5-HT in *Schistosoma mansoni* is believed to be host-derived via specific carriers, and in *H. diminuta*, circadian variations in the levels of 5-HT have been documented. (14)

Small amounts of the catecholamines noradrenaline and dopamine have been shown to be present in the cestode *H. diminuta*. (15) Analysis of histamine levels in *H. diminuta* showed that the worm does not synthesize the monoamine, but likely acquires it from its host by diffusion. (16)

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11. Grimmelikhiujzen CJP, Westfall JA. The nervous system of cnidarians. In: Breidbach O, Kutsch W, eds. The nervous system of invertebrates: an evolutionary and comparative approach. Birkhäuser, 1995; pp. 7–24.

4. Gustafsson MKS, Halton DW, Kreshchenko ND, Movsessian SO, Raikova OI, Reuter M, Terenina NB. Neuropeptides in flatworms [Review]. *Peptides* 2002;23:2053–61.

5. Shaw C. Neuropeptides and their evolution. *Parasitology* 1996;113:S35–S45.

9. Halton DW, Gustafsson MKS. Functional morphology of the platyhelminth nervous system. *Parasitology* 1996;113:S47–S72.

12. FitzGerald MJT, Gruener G, Mtui E. Clinical neuroanatomy and neuroscience. 5th ed. Elsevier Saunders, 2007.

13. Gustafsson MKS, Nässel D, Kuusisto A. Immunocytochemical evidence for the presence of substance P-like peptide in Diphyllobothrium dendriticum. *Parasitology* 1993;106:83–9.

14. Mansour TE. Serotonin receptors in parasitic worms. *Adv Parasitol* 1984;23:1–36.

15. Chou T-CT, Bennett JL, Bueding E. Occurrence and concentration of biogenic amines in trematodes. *J Parasitol* 1972;58:1098–102.

16. Yonge KA, Webb RA. Uptake and metabolism of histamine by the rat tapeworm *Hymenolepis diminuta*: an in vitro study. *Can J Zool* 1992;70:43–50.

Evidence exists for the amino acid transmitter glutamate as a neurotransmitter in at least two cestodes. (17,18) GABA is an important inhibitory neurotransmitter of widespread occurrence in both vertebrates and invertebrates. There is widespread occurrence of GABA in the CNS of all flatworms examined and a much higher concentration of the amine has been revealed in the cestode. (19)

BIOLOGICAL PROPERTIES OF TAENIID SPECIES

Even though the different taeniid species have many physiological functions in common, they may differ in tissue tropism, the ability to reproduce asexually inside the metacestode, the ability to establish new metacestodes after rupture of the original metacestode, and different parasite-induced trophic transmission (PITT) effects.

Tissue tropism of different taeniid species

Different taeniid species have different tissue tropism in the intermediate host. It is unknown in what ways the oncosphere is able to selectively increase its chances to establish a metacestode in certain tissues of the intermediate host, but it seems reasonable to assume cell signalling as the responsible mechanism. *Echinococcus granulosus* metacestodes are most common in the liver and the lungs, but may be found in most tissues, including the CNS. *Taenia multiceps* metacestodes are usually found in the brain and spinal cord, but may be found in the subcutaneous tissues as well. (20) *Taenia serialis* metacestodes are usually found in the connective tissues. (21) The metacestodes of the most common dog taeniid, *T. hydatigena*, are usually found in the abdominal cavity and in the liver. *Taenia pisiformis* and *T. crassiceps* metacestodes have tissue tropism similar to that of *T. hydatigena*, whereas *T. ovis*, *T. krabbei*, and *T. cervi* metacestodes seem to have tissue tropism for muscle tissues. The metacestodes of the cat taeniid, *T. taeniaeformis*, are usually found in the liver. The tissue tropism of the specific taeniid metacestode will obviously be of great importance for the sort of disease that the metacestode will cause.

Ability to reproduce asexually inside the metacestode

The well-known *T. solium* metacestode contains just one protoscolice and is unable to reproduce asexually inside the metacestode. In contrast to those of *T. solium*, the metacestodes of several other *Taenia* spp. and *Echinococcus* spp., for instance *T. multiceps* and *E. granulosus*, are able to reproduce asexually. The lifetime of the *T. solium* metacestode is supposed to be just a few years (22), while the *E. granulosus* metacestode

17. Keenan L, Koopowitz H. Physiology and in situ identification of putative aminergic neurotransmitters in the nervous system of Gyrocotyle fimbriata, a parasitic flatworm. *J Neurobiol* 1982;13:9-21.

18. Thompson CS, Mettrick DF. The effects of 5-hydroxytryptamine and glutamate on muscle contraction in Hymenolepis diminuta (Cestoda). *Can J Zool* 1989;67:1257-62.

19. Eriksson KS, Maule AG, Halton DW, Panula P, Shaw C. GABA in the nervous system of parasitic flatworms. *Parasitology* 1995;110:339-46.

20. Garcia LS. Diagnostic medical parasitology. 5th ed. ASM Press, 2007.

21. Taylor MA, Coop RL, Wall RL. Veterinary parasitology. Blackwell Publishing, 2007.

22. Pawlowski ZS. Taenia solium: basic biology and transmission. In: Singh G, Prabhakar S, eds. Taenia solium cysticercosis: from basic to clinical science. CABI Publishing, 2002; pp. 1-13.