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Professor Paul Gottlob Layer

Research achievements

This section sketches some of our research goals which could be achieved only thanks to excellent co-workers and students.

1. **Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) as non-neuronal & non-enzymatic cholinergic players in embryogenesis.** Modifications of the Karnovsky-Roots enzyme histochemistry and their extensive applications in the early chicken embryo led to detailed comparative analyses of AChE and BChE expressions in the entire embryo. Spatial and temporal distinctions between expressions of both types of cholinesterases (ChEs) were revealed. Considering their early (pre-neural) expressions, embryonic functions of embryonic ChEs as had been postulated by U. Drews (Univ. Tübingen) in the 70ies were extensively substantiated in different organ systems of the developing avian embryo, i.e., BChE was established as a proliferation marker and AChE as a postmitotic differentiation marker. Notably, BChE as a transiently expressed stem cell-associated player in development was brought into focus (publ. in PNAS, Development, J. Neurosci. and others; independent research at MPI Tübingen/Germany, and at Technical University (TUDa) in Darmstadt/Germany). This cholinergic research work was devoted to development of the neural tube, retina, brain vesicles, rhombomeres, cranial nerves, trunk, somites, appendages and bones in chick and mouse embryos.
2. **Non-enzymatic adhesive actions of cholinesterases** (e.g., non-cholinergic) were documented in an *in vitro* neuronal assay system for the first time in a seminal paper of 1993 (Layer et al. 1993). Complex formation of AChE with laminin can contribute to adhesive functions (Paraoanu et al. 2004). Research from foreign laboratories had established that ChEs belong to a family of cell adhesion molecules (CLAMS), including e.g., neuroligins and neurotaxin, which all but ChEs do not exert any ChE enzymatic activity. Thus, our findings were strongly supported by CLAM research, and had opened the field of ChEs acting non-enzymatically in adhesive cell mechanisms.
3. Cholinergic actions of **cholinesterases on bone development** were established (review doi: 10.1016/j.intimp.2020.106405). Nicotine smoking, but also exposure to pesticides, nerve gases or medical drugs point to the significance of cholinergic effects on bone status. Our *in vivo* and *in vitro* studies on bone development of chick and mouse, including i) expressions of cholinergic components (AChE, BChE, ChAT) in chick embryo, ii) characterisation of defects during skeletogenesis in prenatal ChE knockout mice, iii) loss-of-function experiments with beads soaked in cholinergic components and implanted into chicken limb buds, and finally iv) the use an *in vitro* mesenchymal 3D-micromass model that mimics cartilage and bone formation, revealed complex **crosstalks** of **cholinergic, radiation** and **inflammatory** mechanisms. Thus, cholinergic actions in bone development are driven mainly by classic cholinergic, but also include ACh-independent cycles.
4. **Heralding Tissue Engineering: Regeneration of retinal tissue *in vitro* from stem cells.** Following Moscona´s and Steinberg´s approaches of reaggregating dispersed cells from the embryonic avian retina from 1980 onwards, we have introduced co-reaggregates of retinal cells with cells from the pigmented retinal epithelium (RPE). In contrast to reaggregates from retinal cells alone, in co-aggregates highly organized and normally laminated tissue structures were reconstructed *in vitro*. By choosing the culture conditions, inverted and correctly laminated retinal structures in 3D-reaggregates could be produced (we introduced the term *retinospheroids*; i.e., retinal spheroids). To the best of our knowledge, this was the very first

demonstration that (and how) reconstruction of organized retinal tissue from dispersed stem cells is - in principle - feasible *in vitro* (Vollmer et al. 1984; e.g., Tissue Engineering). These tissue organizing effects were further analyzed in much detail, e.g., addition of Müller glial cells (MCs) and growth factors provided with the medium induced defined cell organizing effects. The cellular mechanics of retinal tissue formation *in vitro*, such as radial cell column formation from proliferating precursor stem cells and their lateral stabilization by MCs, photoreceptor differentiation and sublamination of synaptic plexiform layers (IPL) were investigated. These findings were extended to a murine system (Mongolian gerbil), including cholinergic and Wnt actions in retinal tissue formation.

5. **Growth factors in tumour cells, retinal differentiation and Tissue Engineering.** Studies of binding of nerve growth factor (NGF) with its receptor on PC12 tumour cells, including its dimerization, internalization and degradation contributed to understanding of NGF actions (publ. in JBC, J. Neurosci., and others: postdoctoral work at Stanford/CA). Applying various retinal spheroid systems, actions of FGF2, PEDF, GDNF, BDNF, NGF, insulin and combinations of them on cell type determination and differentiation, e.g., rods and cones, was analyzed (work at TU Darmstadt/Germany). Note: We predicted the future relevance of retinal spheroid studies to Tissue Engineering (human TE) already in the 90ies, when iPSCs were not yet available (work at MPI Tübingen/Germany & TU Darmstadt/Germany).
6. **Photoaffinity labelling of biologic systems.** First introduction and successful application of a photoaffinity label to a biologic system by using an azido-labeled cholinergic radioligand. Both nicotinic acetylcholine receptor (nAChR) and acetylcholinesterase (AChE) from *Torpedo cal.* tissue were selectively labelled at defined subsites of both molecules. Thus, having documented the applicability of photoaffinity labeling by these seminal studies, photolabeling became a valuable technique in cell/molecular biology (publications in PNAS & Mol. Pharmacol., Ph.D. work in Konstanz/Germany, 1973-1976).
7. **Evolutionary Theory.** The post-genomic era questions neo-Darwinian genetic determinism, and thus the *Standard Evolutionary Theory* (SET) needs an extension to EET (Extended Evol. Theory). Instead, open aspects of macroevolution become intelligible by *Evo-Devo* research. At all developmental levels, self-organization acts robustly towards “wholeness”, as exemplified by organoid technologies. In retinal reaggregates histotypical features are reached along different histo-formative routes. Thus, tissue formation is not merely gene-directed, but channeled by unpredictable external conditions. These insights restrict conceptions of onto- and phylogenesis, e.g., both are neither characterized by unlimited randomness nor by finite *genocentrism*. Reinspection of Driesch´s *drive to wholeness*, e.g., *robustness* and *intentionality* appears timely, while his *teleologic* postulates remain undecidable by reductionist reasoning (Layer PG (2013) In: Ann Hist Philos Biol 16, 153-170, ISSN 1863-0197; Layer PG (2021) Naturwiss. Rundschau 74(5), S. 228-237; Layer PG (2022) BIOCOSMOS 1, 12-25. DOI: 10.2478/biocosmos-2022-0002).
8. **Science Philosophy.** See List of Commented Publications; i.e., Layer PG (2005). Vernünftiges und vernünftig Vermutetes zu Gehirn, Geist und Gott. In: Arnoldshainer Texte No. 133, ISBN 978-3-89846-494-9. S. 134-161; Layer PG (2007). Was ist Leben? - Von Zellen und anderen Lebewesen zwischen Genkonstanz und Umweltvarianz. In: Arnoldshainer Texte No. 136, ISBN 978-3-89846-494-9, S. 102-116. Layer PG (2022). Wirklichkeit, Wissenschaft und Ethik in Notzeiten. In: Freies Christentum. 74(5), S. 115-129.