Fifty years of research in Neuropathology

*Scientific Biography of Johann Michael Schröder (*1937)*

Content and Summary

Prologue

Home at birth in Hamburg, Germany, 1937. The eagles on top of the roof were supposed to symbolise the rise of the German empire around the year 1900 when the house was built

Sometimes European people at the age of 80 years such as Johann Michael Schröder (JMS) say: "I am a war child." Then they eat what is left on their plate. Those who survived the second World War (1939 -1945) were not always lucky but they had after an early unconscious and innocent backlash a great rise of fortune behind them. During 70 years of peace after point zero in 1945 everything became continuously better. It was not really 'zero' because there were many occasions for boys to play with. His father bought two milk sheep which provided good nutrition and clothes. The remnants of a house with 51 rooms (Fig. on page 3) and a garden with 5000 m² provided space to live although 50% of the *neoclassical* building with two rising oversized eagles on top of the flat roof were partly damaged by two bombs. Because of the war his father had to quarrel with his brother about leadership in their import and export food company inherited to both of them from their successful father (grandfather of JMS). The one who was determined by the nationalsocialistic administration as the leader had not to go to military service. The other one had to go to war.

Those who came back from the war were in trouble with bombed houses, persecution, hepatitis, food restriction, inanition, etc. The father of JMS lost his wife by divorce because the leading Italian fascist in Hamburg promised her heaven in Italy when he came out of the post war concentration camp. JMS and his brother returned after 3 years of 'evacuation' to a rural farm following the Anglo-American bomber attack "Gomorrha" on the city of Hamburg producing the famous firestorm. Back in town they had to speak and write 'High German' again after speaking 'Low German' in the state of Schleswig-Holstein during the age period between 5 and 9

years, nearly without intellectual and musical education. This was probably the most serious effect of the war on JMS and his brother: to learn speaking and writing in 'High German' in schools with 30-40 class mates and no one at home who had time to help. Teaching hours were alternating from one school to the other: half of the week in the morning, the other half in the afternoon including Saturdays. No one claimed about psychological problems with the diurnal rhythm etc. At that time Switzerland and Sweden generously provided soup for lunch.

It was not before the father of JMS hired an additional private home teacher providing lessons 2 hours a week that he got to know for the first time, that one could do home work for school continuously over 2 hours. After about half a year there were no school problems anymore, especially in mathematics, latin, greek, music, and gymnastics where his school mates had no advantage from automatic learning in an intact family at home.

When his father married a postgraduate medical doctor, 25 years younger than he, JMS and his brother felt that there was a difference between a mother and a step mother. His brother was supposed to enter, before finishing school, the company which was dealing with food distribution. The company's slogan was "Piek Fein", written on the base of an impressive pyramid as trade mark. No one took really care of the boys' higher education.

Before and during the war their father had had success without finishing school or academic graduation. This was propagated during Hitler's regiment; self made men were *en vogue*. But after the war it was difficult for him. Nevertheless JMS got financial support from his father to study and to pass examinations in school and medicine including the medical doctorate. For one semester only he got support from the state for one of the universities to where he was going: Freiburg/Breisgau, Munich, Vienna and again Munich. The father's post-war business went down continuously. There was no way to compete with big food companies. JMS however felt that there was some discrepancy between the representative house built before World War I and II, now partly damaged but beautifully situated above the river Elbe compared to the moderate monthly support that he received. Finally the *old* house was replaced by a modern one with 8 flats hardly avoiding bankruptcy while JMS was studying medicine elsewhere.

The mother of JMS was in Italy unable to provide support for the boys from her new husband who continuously succeeded with an import and export company for wine and fruit juices. Not before their mother inherited remnants of a fortune from her mother that her mother had inherited from her father who had been a 'Geheimer Kommerzienrat' (Carl Funke 1908) in the "Rheinischer Provinziallandtag" etc., and who before inheriting cool mines from his father, a constructor of Krupp's 'Villa Hügel' etc., took over further industrial positions and finally the position as supervisor of the board of advisors ("Aufsichtsratvorsitzender") of the "Essener Steinkohlenbergwerke A.G.". His fortune increased during the First World War but after World War II vanished because cool mines in China operated much cheaper and the oil industry took over nearly everything.

In conclusion: It appears that JMS had to start from point 'zero', founding a new division of neuropathology in Mainz (1974 – 1981) (see below) and thereafter a new department of neuropathology in Aachen (1981 – 2004) (see below), hardly comparable to the positions of some of his ancestors.

Chapter A. Doctoral thesis ('Localisation of ammonshornsclerosis in an arterial border zone). Munich, Germany, 1962

The doctoral thesis of JMS in Munich was explicitly not proposed to gain scientific progress but as an introduction to science by which a specific theme was studied, in his instance: two autopsy cases with 'ammon's horn sclerosis, from a new light microscopic point of view. One of these cases and some other ammonshorns, serially sectioned and carefully documented with an impressive number of light microscopic photomicrographs by Bodechtel (1930) showed that the main lesion was located at the border between the territory of the anterior and posterior cerebral artery, falling into the category of arterial border zone infarcts. This correlation had not been recognized before.

During his subsequent life as neuropathologist while cutting brains he is said to have macroscopically screened every ammonshorn, but never detected another lesion with its greatest extent just behind the uncus where the arterial border zone of the largest intracranial arteries is located. Yet this experience does not mean that this hypothesis was invalid and unimportant. When trying to revise the original specimens of Bodechtel which supposedly were stored in the archive of the Kraepelin Institute of the Max Planck Society in Munich, JMS was noted by the director of the institute, Professor Dr. Willibald Scholz himself, who happened to show up at the archive's entrance. Scholz kindly indicated that the archive was not open to the public. Later he called up the 'doctor father' of JMS, Professor Joachim-Ernst Meyer, Scholz's former student, arguing about the uncontrolled activity of the student. Thus the first attempt of JMS towards original research was blocked and one wonders whether the doctoral thesis would have gotten a better note than 'cum laude' when JMS would have been allowed to work up the larger series of archival cases. Nevertheless when later JMS applied for a position at Zülch's institute in Cologne (see Chapter B) he appeared to be welcome; one of the reasons may have been that cerebral border zone infarcts were in the center of Zülch's interest in 1963.

Chapter B. Medical and Scientific Assistant ("Medizinalassistent"; "Wissenschaftlicher Assistant") at the Max Planck Institute (MPI) for Brain Research, Department of General Neurology, in Cologne, Germany (Director: Prof. Dr. med. Klaus Joachim Zülch). Military Service: 1963-1965; *9 articles*

Writing the first case report on a cavernous hemangioma, of the spinal cord published in 1964 at the Max Planck Institute for Brain Research in Cologne, Germany

Here, at the "Max Planck Institut für Hirnforschung, Abteilung Allgemeine Neurologie" contrary to his expectations, JMS did not become involved in the study of border zone infarcts (cf. Chapter A). Now brain edema of the white matter was in the center of interest. The lesions were microinfarcts in cats experimentally induced by injection of Hostalen PP microemboli, about 30-37 µm in diameter, into the carotid artery. JMS took advantage of the extensive experimental light microscopic series of Zülch with Tzonos to analyse and publish an article on the chronology and manifestation time of microinfarcts in cat brains, the lesions covering time intervals between 30 minutes and three months after the injection (Schröder and Tzonos 1967). The experiments afforded special skills to prevent rupture of the carotid artery and microembolic or ether overdose.

Another experiment at Zülch's institute, introduced by Wolfgang Wechsler, focussed on electron microscopic analysis of traumatic brain oedema in mice. In 1963 the new, large, and open field of electron microscopy had opened worldwide for a multitude of applications. Thus JMS achieved some expertise with electron microscopic techniques (Schröder and Wechsler 1965). One of their papers written in German hypothesized disturbances of 'adhesion power' between cells in the oedematous cortex versus that of myelinated fibers in the underlying white matter.

Based on these studies, JMS successfully applied for a one year grant provided by the Max-Kade Foundation conveyed through the German Research Foundation (Deutsche Forschungsgesellschaft, DFG). This opened the way to American research in medicine at a famous institution: the Harvard Medical School (HMS) (see below: Chapter C). Head of the Neurology Department was Ray D. Adams, Bullard Professor of the HMS, who happened to be a friend of Klaus Joachim Zülch, Cologne, Germany. In Adams' Department was a pioneer of electron microscopy, especially of its application to peripheral nerves, Henry deF. Webster, MD, and Associate Professor of Neurology at the HMS. He had succeeded in getting a big grant for a small yet fully equipped electron microscopic laboratory at the Massachusetts General Hospital ("MasGeneral", MGH) provided with a Siemens electron microscope. This electron microscope was more sophisticated than the Zeiss 9 electron microscope in Wolfgang Wechsler's lab at Zülch's Department in Cologne. Again the doctoral thesis, appropriate age, initial experience with electron microscopy, and the connection of Zülch to Adams helped to get this grant. JMS hoped that his previous experimental studies on the manifestation time and chronology of brain infarcts would recommend him to assist in the study of Webster and Ames on the chronology of changes in the retina of rabbits *in vitro* following oxygen and glucose deficiency either selectively or combined. Interestingly these electron microscopic studies were combined with electrophysiological recordings. But the window opened at another scene (see Chapter C).

Military service: After the decision that JMS could go to Boston there were 7 months which he used to follow his duty as "Restant", someone who because of his indispensibility in doing specific research projects was 'set back' for later use in the army. He needed 6 months for the service and one month for voluntary military practice to become Medical Officer in Reserve ("Stabsarzt der Reserve"). Thus he did military service in the biggest and best equipped Central Military Hospital of the Bundeswehr in Koblenz, Germany, not far from Cologne. Due to recommendation of Zülch at the well recognized MPI (see above) he could do his service at the Neurologic-Psychiatric 60-bed Clinic of the Hospital using it for his future career in neuropathology.

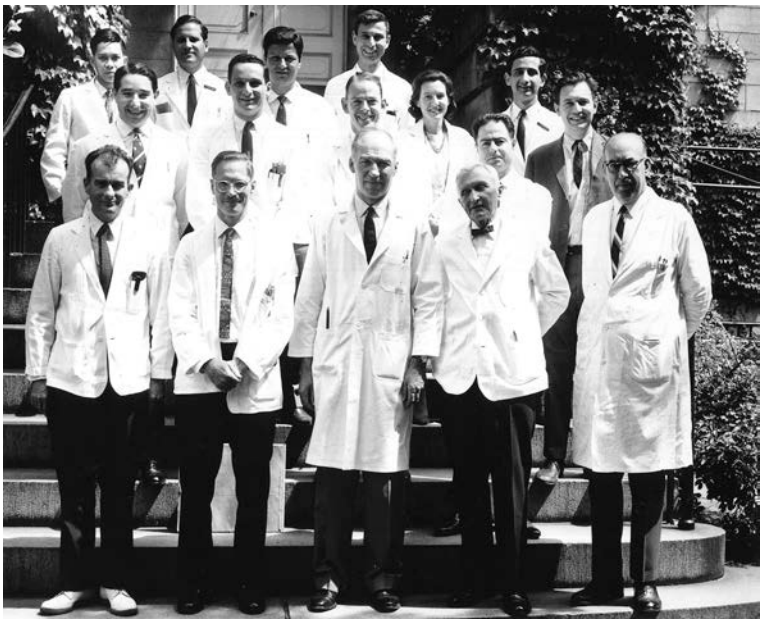
There he had, among other clinical tasks, to perform neurological and psychological tests to identify soldiers simulating psychiatric illness to be freed from military

service, or others who were really ill to be dismissed from the army. Most impressive were mid-life sergeants who had become alcoholics because of the boring service. Most impressive was a fully developed delirium tremens. Whenever he needed something like reprints or other material he could order it in the office where there were two sergeants waiting for something to do answering: “Jawoll, Herr Stabsarzt...”

For the Annual Festivity (“Standortfest”) of this big military hospital JMS was not allowed to come and dance with a girl friend. It had to be a fiancé what after 6 years finally turned out to be the truth. But the time passed by and he had received an impression of the social and military life of an army in peace.

Chapter C. Research Fellow for one year at the Harvard Medical School (HMS), Boston, Mass, USA. (Chief of the Department of Neurology and Bullard Professor at the HMS: Ray D. Adams, Fig. on page 10); Head of the Electron Microscopic Laboratory at the Massachusetts General Hospital ('MasGeneral') and Associate Professor at the Department of Neurology of the HMS: Henry deF. Webster, Fig. on page 10): 1965/6; *4 articles*

Arrival in Boston: After his arrival in Boston JMS was invited to stay two nights at Henry ("Harry") Webster's home.



Senior Neurologists at the Harvard Medical School in 1966: in the lower row, first from the left: Henry deForest Webster; third from the left: Raymond D. Adams. Upper row, third from the left JMS: Research Fellow

Harry (Fig. on p. 10) liked to abbreviate his second first name from "Deforest" to "deF." (as his wife, Marion, explained to JMS). She was born in Hungary and knew to speak German but did not make use of it. She took care of him the first two days before JMS found a nice apartment in Cambridge on the other side of the river Charles. He remembered to have felt guilty because he did not make up his bed after the first night because he thought that such a prominent family presumably had adequate personal to take care of things like that. But this was not the case.

A couple of days after his arrival on October 1, 1965, at the HMS, Ray D. Adams, the highly respected and famous Bullard Professor of the HMS and Chief of the Neurology Department (Fig. on p. 10), 'dropped' into Harry's lab asking JMS whether he would like to translate a paper into German which Adams had prepared as a

manuscript in English. Adams was going to read it as an invited platform presentation on myotonic and paramyotonic muscle diseases at the International Muscle Congress which Erich Kuhn was going to organize on December 4, 1965, in Heidelberg, Germany, the University where Wilhelm Erb, the famous myologist, had been working. The meeting took place at the occasion of Erb's 125th birthday. JMS translated the manuscript during the following weekend and got 50 Dollars in cash, the first and last black money that he earned in the USA. He remembered that he had some trouble to translate "significant" which in German is only used in connection with statistics. After the meeting Adams recalled that Kuhn appreciated his remarkable knowledge of the German language. Yet Adams presented his paper in English, while delivering the manuscript for reproduction in the well edited congress volume published one year later with my German text including the erroneous translation of significant into 'signifikant' (Adams, R. D. in: E. Kuhn, Ed., Springer 1966, P. 191-203).

Conferences: The conferences at lunchtime were of highest interest. It seemed that whenever anybody in the world had something new and important concerning neurosciences to present he tried to get a chance for discussing it at the Neurology Department of the HMS. Thus JMS got to know many European and other neurologists or specialists such as P. K. Thomas or John Walton within a single year, including his former chief, Klaus Joachim Zülch. While the staff had their lunch, the invited speaker tried to keep people awake. This was even more difficult on Thursday afternoon sessions. The number of residents trying not to fall asleep was inversely related to the interest and perfection of the presentation.

Slide sessions: A speciality at Adams' department were microscopic sessions where residents in training could study original slides from interesting neurological autopsy cases. Adams asked everyone for his opinion in a calm, yet concentrated atmosphere. So JMS was asked already during his first participation at such a session. He realised that his extensive Greek lessons, 6 hours a week during the last 5 years at school, a traditional humanistic gymnasium in Hamburg, Germany, were of little help, though not totally in vain. At least was he able to explain many terms in clinical medicine which were strange to his highly trained American colleagues. His English was based on years of only two hours a week in School, reading, e. g. Sommerset Morgham loudly one by one for about 10 min each.

Center of interest: One of JMS' greatest surprises at the HMS was that not therapy stood in the center of interest but pure science aiming at essentially new observations presented in the highest state of the art.

Use of the electron microscope

At that time an optimal level concerning ultrastructural preservation of pathological tissue was achieved in Harry's lab when JMS arrived although for the first three months he had to cut nerves which were resistant to ultrasectioning. Glass knifes were insufficient and the epoxy mixtures for embedding were too soft. Some diamond knifes produced terrible scatter instead of smooth and even ultrathin sections. After some months these problems were solved and disturbing artefacts could largely be eliminated. The thickness and thus the contrast of the ultrathin sections could be regulated by the improved, then perfect diamond knives combined with optimized staining. By using as little as possible electron beaming of the area of interest 'melting' of membranes and other structures by the electron beam was prevented as good as possible. Any astigmatism of the electron beam had always to be corrected in the Siemens electron microscope by carefully cleaning and centralization of the 20 µm diaphragms in phosphotuncstic acid and optimal focusing of the electron beam at high vacuum in the center of the electron microscope.

Publications

During the year in Harry's lab at the HMS JMS was not distracted by clinical or family duties except for a few visits to a grand uncle living in Park Avenue, New York, USA. The situation provided a chance to concentrate on research work. Finally there were 4 publications that formed the basis for future activities, and, what should be mentioned already at this point: considerable success.

(1) Formation of so-called onion bulbs in peripheral nerves: As already mentioned, after three months of experience, JMS finally succeeded in using the electron microscope and the ultramicrotomes for cutting semithin sections and ultrathin sections from epoxy-embedded nerve specimens. Now excision, fixation, and embedding had also been optimized at Harry Webster's lab. JMS came out with optimal electron microscopic images using 6.5 x 9 cm glass negatives which were carefully screened with a hand magnifier before being printed at a magnification of

3.5 times on large 18 x 24 cm prints. A professional Russian photographer was employed in Harry's laboratory who for publications produced series of photographs by graded shades of grey and contrast after carefully selecting the optimal grade of the photo paper and using a scaleograph for the area to be published.

In summary, electron microscopic identification of Schwann cells forming so-called onion bulbs in hypertrophic neuropathy was the result that JMS was able and allowed to present as the only author at the Meeting of the American Society of Neuropathology in Washington, DC, in 1966. In addition, he counted the fibroblasts that were increased in numbers and had apparently added to the prominent increase of endoneurial collagen leading to focally accentuated pseudohypertrophy of nerves.

By the way, it was remarkable that a female patient could be persuaded to offer an additional, the fourth, hypertrophic nerve segment, in her case the N. auricularis major, for achieving optimal fixation, embedding, and electron microscopic images of her nerves for 'flawless' publication in accordance with Harry Webster's standards (Webster et al. 1967). It was Arthur Asbury who had been able to persuade her. As an ethical excuse it should be emphasized that her nerves were so sick and thick that their function must have been lost.

(2) Ultrastucture of muscle fibers in myotonic dystrophy: A somewhat analogous procedure was used to get electron micrographs from muscle biopsies of Dr. Adams's cases with myotonic dystrophy, myotonia congenita, and paramyotonia congenita. Careful selection of the muscles for a biopsy, precise technique for excision and fixing a 2 mm thick and 2 cm long piece of muscle by two sutures to a wooden stick for optimizing longitudinal and cross sectional orientation was a prerequisite for optimal fixation in phosphate-buffered 6 % glutaraldehyde solution as elaborated in Andrew Engel's lab at the Mayo Clinic in Rochester as had been told. Careful orientation during embedding in especially designed rubber molds finally granted an optimal result. Ray Adams was co-author and JMS became rather well known by this paper (Schröder and Adams 1968). Interesting proliferation and enlargement of terminal cisternae were detected as well as proliferated transverse tubules, abnormalities of myofibrils such as ring fibers and sarcoplasmic masses with destructed myofibrils, and increased numbers of abnormal nuclei.

(3) Biochemical findings concerning the sarcoplasmic reticulum (Samaha et al. 1967) were also published in cooperation with this study although no significant substrate of myotonia was detectable. It took more years to identify altered ion channels causing these diseases.

(4) Fixation of the central nervous system: A paper with Descarries (Descarries and Schröder 1967, 1968) emphasizing large amounts of clean distilled fixative for optimal preservation of the central nervous system did not reach similar recognition such as the articles mentioned above on hypertrophic neuropathy and myotonic dystrophy.

Thus JMS had published finally 4 papers that came out of this one year stay at the HMS; this was rather unusual at that time for a research fellow from across the Atlantic or anywhere else.

Off note: Concerning publication of results JMS was surprised that some political arguments played a role: Thus authorship was subjected to selective interests in peripheral nerves or muscle. E. g., JMS was elected as the first author in the article with R. D. Adams on ultrastructural changes in myotonic dystrophy. But he was placed second in the published version of the study on hypertrophic neuropathy although he did most of the work regarding electron microscopy of Schwann cells and counting of fibroblasts. The secret message to a naïve was: Politics are involved even at the highest level of medical science.

At the end of the time in Boston, JMS started his American grand round: 10 000 miles by car in three weeks through the Eastern and central parts of Canada, and mayor sites of the United States from East to West, to South, and back North-East. The first stop was Quebec to visit Larry Descaries, his colleague in Harry Webster's lab. The next visit was at the Mayo Clinic in Rochester, Minn., USA, where JMS by chance met Peter J. Dyck, the famous neurologist whom JMS had briefly met at the Neuropathology Meeting 1966 in Washington, D.C., after he had presented the paper on the formation of 'onion bulbs'. In fact, Peter Dyck happened to be at the Mayo Clinic and kindly took his time to show the essentials of the famous Clinic which was already worth the long tour. Finally JMS was glad to be back in Boston. At that time events at the HMS were more interesting to him than the gigantic scenery of the country.

Harry Webster was on the leave to Miami when JMS left Boston after one year. A larger and similarly equipped laboratory, also with a Siemens electron microscope, 'was waiting' for JMS in Frankfurt am Main, Germany. Karl Aström from Stockholm, Sweden, took over Harry's lab in Boston.

Plans for the future

When working over the draft of the manuscript on myotonic dystrophy with Ray D. Adams, JMS consulted him about what he should do regarding his obligation to return to Zülch's department in Cologne, Germany. The alternative was an offer to be in charge of the electron microscopic laboratory in Krücke's Department of Neuropathology at the Max Planck Institute for Brain Research, Frankfurt a. M., Germany. Adams argued that Krücke was considered the best recognized neuropathologist in Germany.

In case of returning to Cologne, JMS would have been subordinated to Wolfgang Wechsler who was in charge of the electron microscopic laboratory there. Before going from Cologne to Boston JMS had been contacted by Krücke already at the International Congress of Neuropathology in Zürich in the year 1964. There Krücke had offered JMS a position as "Wissenschaftlicher Assistent" in charge of the electron microscopic laboratory at his institute in Frankfurt/M., Germany. As a conclusion Adams recommended to join Krücke as did already Hermann Hager. Hager had published with Wolfgang Wechsler electron micrographs on denervated skeletal muscle of rats at the Kraepelin Institute (MPI for Psychiatry) in Munich. This inspired JMS' early ideas regarding research on neuromuscular endplates which in fact were much later approached by his studies on muscle spindles (see Chapter E and F).

Ray Adams' policy at the HMS was obviously that someone who had worked at his department should follow the line and should not drift somewhere into peripheral practice. In this context, he mentioned: "In earlier times we (the Americans) came to Europe, now you (the Europeans) come to America." And continuity was what JMS wanted as well.

Krücke was in charge of two positions: as Director of the Department of Neuropathology at the MPI for Brain Research in Frankfurt/Main, and as Director of the Edinger Institute of Neurology at the Johann Wolfgang Goethe University in Frankfurt/Main, Germany. JMS' suggestion was that someone who had achieved such

a ‘double director’s position knows how to get ahead and should be able to show the right way. In addition he had published seminal articles on the pathology of peripheral nerves. His ‘secret’ was simply: “One has to do good work.” In any case, in Krücke’s department experiments were running where a trained electron microscopist would have optimal conditions and excellent material to work with.

Late spin-off effects of the reseach fellowship at the HMS: books

Myotonic dystrophy (Schröder and Adams 1968), and another paper on congenital myotonia and myotonic dystrophy (Samaha et al. 1967) etc. opened the way for writing a standard book on muscle diseases (“Pathologie der Muskulatur”, Schröder 1982; 813 pages) in the actual series of German books on “Special Pathologic Anatomy” (see below, Chapter D and E).

The initial price for a single volume of this book in this series was 660 DM (approximately 300 US$ at that time), for its later available ‘soft cover edition’ of December 12, 2011, it was 54.99 EURO. This shows how the (financial) value of medical books may vanish. However, according to the German National Library an online edition advertised for a while by Amazon was also available.

Seventeen years later followed another standard book in the same series, this time on “Pathologie peripherer Nerven”, also in German which, however, was not finished before 1999 (Schröder 1999; 862 pages, 1052 Figures; available as soft cover edition since November 21, 2012).

An atlas with only 380 pages but using the same figures followed in 2001 in English: “Pathology of Peripheral Nerves. An Atlas of Structural and Molecular Pathological Changes”, again written by JMS, now in English by himself because there was no one who would have liked to take over the work of translation.

The price of the original edition in 1999 was 870 DM. Now it is offered by Amazon for 300 EURO, a soft cover reprint since October 21, 2012, for 93 EURO. An online edition is also available according to the German National Library. The author, JMS, had not been consulted about these reproductions; he did not even get a free issue.

For both books, “Pathologie der Muskulatur” and “Pathologie peripherer Nerven” written in German did JMS insist on writing the book alone, without a co-author. It came to know that an author in another series of the Springer Company, e. g.,

"Handbuch der Neurochirurgie", let co-authors wait for up to 14 years for his contribution. Finally, authors who had finished their part many years before, went to court. Therefore JMS asked the editor to confirm that forensic pressure would be excluded. Nevertheless soft pressure by reminding him year by year did occur, in the case of "Pathologie der Muskulatur" from 1971 to 1982.

A paperback on a proposed German nomenclature of neuromuscular diseases appeared as a result of a series of annual meetings of a large committee in Heidelberg, Germany (edited by Schröder, Hopf, Wagner and Amelung 1989). This is also included in the list of books in the German National Library.

JMS' contribution (110 pages) to an additional book edited initially alone by Bernd Neundörfer is in the National Library documented as authored equally by both. Nevertheless this authorship may be considered as an honour because it is registered in the series of 'exil collections' of books although JMS had not been exiled.

Further spin-off effects

Based on the final C4 professorship in Aachen since 1981 (see below) and the book on Pathology of Muscle Diseases appearing in 1982 (see above), JMS was elected as 'Head' ("Leiter") of the "Neuromuscular Reference Center of the German Society of Neuropathology and Neuroanatomy" in 1983, and closely thereafter also as Member of the Executive Committee of the Research Group of Neuromuscular Diseases of the World Federation of Neurology headed by John Walton, Newcastle upon Tyne, GB.

Chapter D. Scientific Assistant ("Wissenschaftlicher Assistent") in charge of the electron microscopic laboratory, Max Planck Institute (MPI) for Brain Research, Department of Neuropathology, in Frankfurt am Main, Germany (Director: Prof. Dr. med. Wilhelm Krücke, also Director of the Ludwig Edinger Institute of Neurology of the Johann Wolfgang Goethe University, Fig. on page 18): 1966-1974; *25 articles*

‚Brain cutting' performed by Masaya Oda as a guest before he was leaving the department while Professor Wilhelm Krücke, on the right was watching. Usually he did the cutting himself.

Leaving Harvard Medical School, Boston, Mass., USA, after one year JMS was able to continue work on the fine structure of peripheral nerves and muscle in Frankfurt am Main, Germany, at Wilhelm Krücke's Department of Neuropathology of the Max-Planck-Institute of Brain Research, associated in personal union with the Neurological (Edinger) Institute of the Johann Wolfgang Goethe University, in 1966-1974. At that time the MPI was well equipped with everything that was considered necessary whereas the Edinger Institut had only an annual budget of 20 000 DM. Nevertheless the latter formally provided access to the facilities of the University including "Habilitation" etc. (see below).

Regarding continued activity on muscle biopsies JMS was contacted by Erich Kuhn (Chapter C), Internal Clinic of the University Hospital in Heidelberg, for individually taking care of the first open muscle biopsies in Germany that he was taking from his myopathy patients. Several articles came out of this co-operation including experimental work on diazacholesterol induced myotonia. Yet it disappointingly turned out that electron microscopy was not the right method to identify the underlying defect causing myotonia (Schröder and Becker 1972; Kuhn et al. 1979).

Habilitation

Anciennity dominated in Krücke's department. Two colleagues of JMS were first. Thus he had to wait for nearly five years following his arrival 1966 in Frankfurt. It took about two years for each of them to finish their *opus magnum*.

Writing in German: Because JMS needed papers to be used for the prospective habilitation (see below) he wrote major articles at that time in German. The impact factor had not started its way of success in the mind of himself and scientists in the sixties and seventies of the preceding century.

'Habilitation machine': Wilhelm Krücke occasionally took his time to look at ultrathin sections of neurofibromas on the green screen in the dark room of the electron microscope with the help of the technician whom JMS had trained to use the EM. He needed images for his article on peripheral nerves in the series of the big Textbook on Neurosurgery for which the co-authors had been waiting for many years. He as mentioned above sardonically called the electron microscope a 'habilitation machine', but an effective one.

Habilitation thesis and lecture: When JMS had to present a lecture in front of the Medical Faculty at the occasion of his Habilitation (Frankfurt/M. 1971) he was not allowed to use any manuscript, slides, or folia. Furthermore it was not allowed to present the same subject as covered in the manuscript of the thesis written selectively for the habilitation procedure. In his case the voluminous manuscript summarized the results of three experimental series, published in 1968 - 1970: (1) isoniazid neuropathy, (2) experimental allergic neuritis, and (3) nerve grafts (for details see below). Because these papers were written in German they could easily be incorporated into the manuscript of the thesis with the title: "Degeneration and regeneration in the peripheral nervous system."

In accordance with the need of a purely oral presentation without figures in front of the faculty JMS selected: "Myotonia: a disease without a structural substrate". It was the hottest summer since many years and there was no air condition. Thus the audience insisted on having the windows opened despite a nearby automatic hammer filling its surroundings with heavy noise. Thereby the audience seemed to fall asleep but woke up when JMS finished. Some intelligent questions were asked

and after polite applause he could leave the procedure with the impression that everybody agreed.

It was custom that one had to visit all inner faculty members personally thereafter. The dermatologist appeared to like the electron micrographs. At least he offered JMS a position at his clinic to install electron microscopy. But (biographically) JMS thought that life is too short to change basic lines and positions if not necessary.

The HNO (neck, nose and ear) professor had a simple question: "What do you want to achieve in the future?" He inferred: "I wanted to become head of a HNO clinic." That is what he in fact had achieved. JMS was amazed by such an easy going career and answered: "I would like to become head of a decent department of neuropathology." That is what JMS finally got; but it was not so easy and took another 3 years (at the age of 37) to become head of a small division as C2-Professor in Mainz, and another 7 years to be appointed as Director and C4-Professor of a larger department in Aachen (see below).

Biopsies

Studying muscle and nerve biopsies came more into the center of JMS' interest during the future time in Mainz and Aachen (see below). In fact starting in Frankfurt/Main his group was obviously pioneering such biopsies in Germany (Thalidomid neuropathy: Krücke et al. 1971). Finally when he was in Aachen (Chapter F) there were nearly 100 neurological clinics in Germany, Austria, and Belgium each submitting 2 to 5 muscle biopsies and/or nerve biopsies per year. However, in no case did JMS ask for a biopsy but he insisted on optimal excision and fixation by anyone who performed any biopsy and who asked for cooperation in diagnostic or scientific projects during the years in Frankfurt/Main, Mainz, and Aachen (Chapters D, E, F) . Unlike colleagues in neuroradiology, co-authorship was implied when electron microscopic or other images were provided by JMS' laboratory to be published. Recognition appears justified concerning electron microscopic techniques and careful photographic elaboration in respect to selection of areas, magnification, sharpness, contrast, avoidance of artefacts, scratches, dirt particles of any kind, or wholes or other defects in ultrathin or other sections.

Experimental work

There were five experimental models which rather simultaneously revealed a large amount of data during the years 1966-1974 in Frankfurt/Main. Some data were left or 'buried' in Proceedings or books without international reputation. The experiments comprised:

(1) Experimental allergic neuritis (EAN) in rabbits
(2) Isoniazid (IHN) neuropathy in rats
(3) Nerve grafts in dogs and patients
(4) Nerves and muscle spindles after nerve crush lesions in rats
(5) Organotypic cultures of rat sensory ganglia following cadmium chloride intoxication

Ad 1: Experimental allergic neuritis (EAN) in rabbits

Pathogenesis of 'onion bulb formation': The work on 'onion bulb' formations started in Boston on biopsies and continued in Frankfurt as part of the study on experimental allergic neuritis (EAN) in rabbits. It revealed that the so-called onion bulbs in peripheral nerves, one of the most frequent and elementary pathological changes in demyelinating peripheral neuropathies, were caused by *'supernumerary Schwann cells'.* These were left over after proliferation following demyelination and remyelination. This pathogenetic conclusion was based on counts of the number of cell nuclei in semithin cross sections, comparing the ratio of nuclei of myelinated nerve fibers in normal nerves to demyelinated and remyelinated ones (Schröder 1968a). There was 1 nucleus per 2.5 remyelinated fibers in EAN whereas in normal nerves this ratio was 1 : 21 for small myelinated fibers and 1 : 35 for large ones. Thus the number of nuclei around de- and remyelinated fibers had increased in EAN by a factor of 8-14.

Similar counts of the number of nuclei were performed on clusters of regenerated nerve fibers in chronic experimental isoniazid (INH) neuropathy (see below), a purely axonal type of neuropathy; here the increase of nuclei per cluster was in the range of 13-30. The higher increase of the number of Schwann cell nuclei per cluster of regenerated fibers in INH neuropathy compared to EAN was obviously due to better preservation of proliferated Schwann cells when there was an increased number of

regenerating axons which could be remyelinated. Repetition of degeneration and regeneration in chronic INH neuropathy was excluded as a possible cause for this higher increase of nuclei in INH neuropathy than in EAN because degenerating fibers were not noted in regenerative clusters.

These results with three large representative electron micrographs and a table documenting the counts were only published in the Proceedings of the German Society of Pathology (Schröder 1968a). This seven page paper was written in German with an English summary. It should have been published in an international, peer reviewed scientific journal because it documents a basic progress in the pathology of peripheral nerves. When P. K. Thomas visited the laboratory at that time in Frankfurt/M. and heard the descriptive term "supernumerary Schwann cells" he agreed and obviously appreciated the value of these experimental results. The role of the basal lamina for orienting the concentrically arranged Schwann cells was also documented.

Our additional, even more important findings concerning the process of *demyelination* in EAN was published with some delay (Schröder and Krücke 1970) because of timely collision with overlapping experiments concerning nerve grafts (Schröder and Seiffert 1970), isoniazid neuropathy (Schröder 1970a, b, c), and results concerning an increase of polyamines in Wallerian degeneration (Seiler and Schröder 1970). Meanwhile Lampert (1969) who had visited the laboratory in 1967 won the run by publishing the pathomechanism of myelin destruction by mononuclear cells in EAN one year earlier. The pathomechanism of demyelination resembled that elaborated by him previously in experimental allergic encephalitis (EAE) (Lampert 1967).

Ad 2: INH neuropathy

INH neuropathy is an ideal, reproducible model of an axonal type of neuropathy. Peroral intoxication by high doses of INH (isoniazid) causes (1) extensive lesions of myelinated (1) and (1b) unmyelinated nerve fibers as well as (2) of the endoneurial connective tissue in sciatic nerves, and (3) alterations of nerve endings in muscle spindles as well as (4) nerve cell perikarya in (4a) spinal ganglia and (4b) anterior horns of rats. But the primary site of the lesion remained uncertain; there were (5) several early changes occurring simultaneously. From the present point of view,

mitochondrial changes may be the first ones. Simultaneous disseminated degeneration and regeneration of nerve fibers (see below: 'hyperneurotization of bands of Buengner') was the most characteristic feature. It was interesting that when writing a chapter on INH neuropathy for the book "Experimental and Clinical Neurotoxicology", edited by Spencer et al. (2000), there were approximately 400 recent articles continuously adding to the discussion of the pathogenesis of INH neuropathy 30 years before (Schröder 1970a, b, c; 2000).

Endoneurial edema (6) and leakage of endoneurial capillaries including endoneurial erythrodiapedesis (7), and (8) enlarged and (8) numerically increased peroxisomes in endoneurial histiocytes were unusual observations. A lathyrogenic effect of INH was discussed in this context. Some unmyelinated axons (9) showed unique deformations. Other differences between Wallerian degeneration and INH neuropathy were also elaborated. There was no doubt that myelin of degenerated fibers was largely digested in Schwann cells (10) which had massively proliferated in bands of Buengner (see above). Removal by macrophages (11), favored by other authors at that time, was less prominent. The regular proliferation of cells (12) during Wallerian degeneration was later used as a model for cell proliferation to study (a) the increase of polyamines (Seiler and Schröder 1970) and (b) the slowing effect of thalidomide on cellular proliferation (Schröder et al. 1995). The main point was, that INH neuropathy is an axonal type of neuropathy less severely afflicting the perikarya although there were vacuoles and irregularities at the periphery of spinal ganglion cells. A lucky ultrathin section revealed even mitosis of a satellite cell (13). This in line with the strong proximal regenerative activity of distally damaged peripheral and central axons underlined the 'dying back' type of the lesion (14).

Pathogenesis of regenerative clusters ('hyperneurotisation of the bands of Buengner'): The regenerating or regenerated fibers were arranged in characteristic clusters of 2 to 15 myelinated and unmyelinated axons. Such clusters had a typical circular shape in cross sections, without showing in serial sections lateral branches to neighbouring clusters. The number of myelin lamellae of the largest fibers measured varied between 24 and 37 over a longitudinal distance of approximately 1 mm (Schröder 1973; Proceedings in: Kunze and Desmedt 1975). This was documented by alternating serial semithin and ultrathin cross sections. Such clusters develop at the site of bands of Buengner using the basal lamina of the original

degenerated myelinated nerve fiber as a scaffold. This was proven by an unique cluster completely surrounded by a single intact basal lamina (paradoxically illustrated in EAN where demyelination dominated, Schröder and Krücke 1970). In INH neuropathy there were usually focal defects in the original basal lamina of the degenerated nerve fiber surrounding and bundling this overshoot of axonal sprouts which obviously represents a special type of safety measure.

Notabene: These regenerative clusters are of diagnostic importance because when occurring in nerve biopsies they are usually diagnostic proof for an axonal type of neuropathy, not of a demyelinating, or neuronal type. The latter tends to be more severe. For instance in alcoholic neuropathy clusters of regenerated fibers tend to be missing (Schröder 1999).

Ad 3: Nerve grafts, neuromatous reinnervation (Minifascicle formation)

An extensive long time (6, 12, 18, and 24 months) experimental study on *nerve grafts in sciatic nerves of 29 dogs* well fixed by perfusion of large amounts (up to 5 liters) of phosphate buffered glutaraldehyde through the abdominal aorta, revealed that short nerve grafts, ca. 3 cm in length, could be of autologous, isologous, homologous, heterologous origin, or even cialit treated ones to be effective in leading at least a large number of nerve sprouts to the peripheral nerve stump (Schröder and Seiffert 1968). Experimental and clinical nerve homografts 6.5 – 10 cm in length, however, were generally inefficient with the exception of a single homograft, possibly an isograft. These findings corresponded to clinical and electrophysiological observations on nerve grafts in 17 patients (Seiffert et al. 1972).

There were interesting 'side effects' coming out of these experiments in dogs; such experiments on peripheral nerves of dogs would in later times probably not have been permitted anymore:

(a) Reinnervation of nerve grafts frequently resulted in a 'neuromatous type of neurotisation' (reinnervation) with multiple minifascicular bundles of regenerated fibers each of which were surrounded by a separate perineurium *('minifascicles')*. The *minifascicles* were named corresponding to the short shirts that were in fashion at that time (Schröder and Seiffert 1970). The term "minifascicles" has become common use in pathology of peripheral nerves since that time. There is even a

special type of neuropathy named “Minifascicular neuropathy” (Umehara et al. 2000).

(b) *Altered ratio between axon caliber (or perimeter) and myelin thickness (or number of myelin lamellae)*: At 6, 12, and 24 months after surgery the best grafts resulted in a large number of regenerated myelinated fibers showing nearly normal axon diameters whereas the myelin sheaths remained disproportionately thin (Schröder 1970, 1972).

(d) Counts of the number of *Schmidt-Lanterman incisures* of teased normal and regenerated myelinated fibers revealed a logitudinal increase of incisures in regenerated ones (Schröder 1974). This was illustrated in *two-dimensional diagrams* based on the number of myelin lamellae counted on electron micrographs, measurements of axonal perimeters, measurements of the internodal length, and counts of the number of incisures per internode in teased fiber preparations.

These *two-dimensional diagrams* of normal and regenerated nerve fibers in rats and dogs had an unfavourable fate in the literature. They were presented (Schröder 1972) and published not in an accessible international journal, but in the proceedings edited 1974 by Hausmanowa-Petrusewicz, two years after the presentation at a congress in Kazimierz, Poland. A brief summary with these two-dimensional diagrams has years later been reproduced (Schröder 1999 and 2000). In essence, the length of the myelin spiral was thought to be unrolled, drawn to scale, and depicted together with the internodal length. The model was analogous to what Hirano and Dembitzer (1967) had illustrated as a *three*-dimensional model which, however, was not drawn to scale. In addition the number of incisures per internode was counted and included in the diagrams. When the first edition of “Peripheral Neuropathy” (Dyck et al. 1975) was going to be printed P. K. Thomas was asked as one of the three editors to add these two-dimensional diagrams to JMS’ article on “Degeneration and regeneration of myelinated nerve fibers”. However, it was already too late to incorporate this piece of information in the ‘big bible’ on peripheral nerves as this opus was sometimes called.

(e) *Dot plots of the axon/myelin ratio* in normal and regenerated nerve fibers were initially performed using light and electron microscopic photographs (Schröder 1970) and later by *optic-electronic video systems and computer programs* adapted for myelinated nerve fibers (‘donuts’) which were said to be “a perfect problem”

(Integramat, Leitz Wetzlar, Germany; Schröder and Seiffert 1972a). Later the optic-electronic system of Zeiss-Kontron (Oberkochen, Germany) was used (see below). By these methods very small myelinated nerve fibers tended to be neglected and myelin folds to be misinterpreted. But the number of fibers, myelin thickness, and axonal diameter as well as their correlation coefficient (*r* value), and myelin area per endoneurial area in percent could quickly be determined applying minor manual corrections.

A useful measure for estimating the degree of reinnervation was, as already mentioned, the estimation of the *percentage of myelin area per endoneurial area in percent*: 33-46% in normal nerves of dogs versus 0-32% distal to nerve grafts in dogs (Schröder and Seiffert 1972). The system was later adapted for evaluating nerve biopsies (see below).

Three-dimensional diagrams were later in Aachen routinely used for indicating simultaneously the number of fibers and their myelin and axonal dimensions (Senderek et al. 1998; Fig. 121 in the Atlas of Schröder 2001). Problems with the resolution power were overcome by manual interference on the screen. These three dimensional diagrams have not become common use elsewhere although they allow rapid estimation of the most important quantitative and qualitative characteristics of a nerve biopsy: number, size, and myelination of fibers which can easily be documented (printed).

(d) The caliber of collagen fibrils at least in some of the minifascicles was thicker than in the surrounding connective tissue indicating a better milieu for development within the minifascicles (Schröder and Seiffert 1970).

(e) *Retrograde atrophy:* The myelinated nerve fibers proximal to insufficient nerve grafts at 6 months showed considerable axonal atrophy whereas the myelin sheaths despite axonal shrinkage appeared relatively 'hypertrophic' and irregular in shape (Schröder and Seiffert 1972). At this relatively early stage degeneration of fibers was not a feature. By contrast, proximal to an amputation neuroma, 44 years after a trauma most fibers had finally degenerated retrogradely (Schröder 1999).

(f) The *main result* of the experiments with nerve grafts was that the length of the grafts is crucial, presumably because of problems with revascularisation. Grafts not longer than about 3 cm may be of any kind. Good results of others with longer

homografts (> 5-6 cm) could neither clinically nor experimentally be confirmed in these studies and would need further investigation before clinical application (Seiffert et al. 1972).

Ad 4: Peripheral nerves and muscle spindles after nerve crush lesions in rats

Nerve crushing in rats: The crush lesion was carefully controlled in respect to the completeness of nerve fiber damage. The regenerated nerve fibers showed after 6 months and one year well developed axons with disproportionately thin myelin sheaths. Yet the normal myelin sheaths in dogs (see above) were thicker than in rats, but the results of reinnervation in respect to the axon/myelin ratio was better in rats than in dogs (Schröder 1972).

Muscle spindles: Comparison of muscle spindles of rats in INH neuropathy after submaximal peroral INH application (Schröder 1970) *versus* Wallerian degeneration after crushing the sciatic nerve of rats (Schröder 1974) revealed some differences. These differences obviously depended on the different stages of degeneration and regeneration studied. Following INH intoxication, there were more numerous acute signs of degeneration of intrafusal nerve terminals such as accumulation of organelles, especially in sensory terminals, with swelling, shrinkage or dissolution mainly of mitochondria.

One and two months after the crush lesion of the sciatic nerve there was already a remarkable *increase of the number of intrafusal muscle fibers* from normally 3-4 to 5-11. This increase was likely not due to splitting of pre-existing fibers but most likely due to maturation of intrafusal satellite cells. At 14 months there were rare atrophic intrafusal muscle fibers filled with small mitochondria or with so-called flecked vesicles. 'Hypertrophic' fibers were regarded as due to erroneous collateral innervation by alpha or beta nerve fibers. Inward widening of the spindle capsule following neurogenic atrophy of muscle spindles was later recorded by Bohl et al. (1980) in Proceedings of the Deutsche Gesellschaft "Bekämpfung der Muskelkrankheiten e.V." not really an international platform. Further transmission electron microscopic (TEM) and Scanning Electron Microscopic (SEM) preparations revealed an additional increase of elastic fibers within muscle spindles (Dieler and Schröder 1990a, b) and a number of three-dimensional aspects of degenerated and re-innervated sensory terminals on SEM figures of teased muscle spindles (Dieler et

al. 1992; Schröder et al. 1989). The isolation of muscle spindles and their preparation for SEM was difficult and had elsewhere not been performed following denervation and reinnervation.

Nevertheless the German Research Foundation (DFG) considered SEM studies on muscle spindles as less efficient than TEM studies. Therefore further investigations of this type were stopped. Another reason for giving up was that Jan Boyd, Glasgow, Scotland, GB, specialized on the physiology of muscle spindles had died while JMS was on the way of contacting him for future cooperation. The efforts to find a disease that was specifically caused by muscle spindles came therefore to an end although later it was thought that certain sensory neuropathies should be associated with specific abnormalities of muscle spindles. These, however, have not been identified thus far. And deficiency of muscle spindles does not appear to be lethal or life threatening.

Ad 5: Organotypic cultures of rat spinal ganglia

Cadmium chloride: An optimal method to study neurotoxic effects of drugs or any other substances such as cadmium chloride independent from blood vessels was to use tissues cultures (*in vitro).* Toxic effects and the pathogenesis of special lesions could be identified without causing pain or any other harm to animals. Arguments of ethic committees or animal protectors were avoided. However, the method needs meticulous precautions: a laboratory with especially clean conditions and sophisticated media for nutrition. This was elaborated for the application of cadmium chloride. Cooperation in fact resulted in optimal electron microscopic images of changes which differed from typical, non-specific tissue culture artefacts (Tischner and Schröder 1972). The main lesions consisted of large focal accumulations of either glycogen or filaments or lipid droplets. In addition spindle-shaped axons and abnormal mitochondria with irregular mitochondrial granules were identified. Changes of mitochondrial granules were presumably related to cadmium uptake disturbing the calcium ion metabolism within mitochondria. The findings indicated that there was a direct toxic effect of cadmium chloride on nerve cells, not only the well-known effect on endothelial cells and blood vessels causing haemorrhages in spinal ganglia and the painful clinical syndrome of “Itai-Itai” disease.

Perspective/Outlook: These experiments formed the basis for later co-cultivation of anterior horn tissue of the spinal cord of rats, together with spinal ganglia, and muscle tissue. The idea was to reproduce a low level reflex arc *in vitro* somewhat similar to that of a muscle spindle with its sensory and motor connections *in vivo* (see below). Unusual and severe mitochondrial and nuclear changes were later identified following application of zidovudine to tissue cultures (Schröder et al. 1992; see Chapter F).

The experimental activities outnumbered the engagement needed for autopsies, neuropathological tumor diagnoses and brain cutting or teaching activities. Nevertheless they were crucial for later becoming appointed as H2-Professor heading a division of neuropathology at the University of Mainz in 1974 (see below: Chapter E).

Chapter E. C2-Professor and Head ('Vorstand') of the new Division of Neuropathology at the Institute of Pathology, Johannes Gutenberg University in Mainz: 1974-1981; *24 articles*

New house in Mainz, Germamy, just finished in 1980 when receiving an offer to start a new Department of Neuropathology with a C4-professorship in Aachen, Germany

The Department of Neuropathology at the MPI for Brain Research in Frankfurt/Main was going to be closed after the retirement of Wilhelm Krücke pending in the year 1975. The themes investigated in his department were thought to be closer to universities than to Max Planck Institutes. Therefore it was an interesting chance for JMS when a lifetime (tenure) H2-professorship at the Johannes Gutenberg University in Mainz (1974) was advertised. There were three competitors: G. Kreutzberg from Munich, F. Matakas from Berlin, and F. Unterharnscheidt from New Orleans, USA. We had to present ourselves officially followed by the usual examination through an executive committee of the Medical Faculty. The document appointing JMS as "Professor and Vorsteher der Abteilung für Neuropathologie" was signed by Helmut Kohl, the later Cancellor of the Federal Republic of Germany, because at that time he was the "Ministerpräsident of Rheinland-Pfalz" where Mainz was the Capital.

Basic Equipment of the Division: The position included being head of a largely independent Division of Neuropathology at the Institute of Pathology including altogether 140 square meters with 1 half- and 6 full-time positions. Additional space and manpower was provided by the Institute of General Pathology for routine work (neurosurgical specimens, especially brain, orbital, cranial, and spinal tumors; cerebrospinal cytology). The position appeared as an initial low step but was open to

the future. And indeed further steps were slowly taken during the next years until a full (C4) professorship was available in Aachen (Chapter F).

Experimental work did not need formal approval at that time. Basically it continued on the following lines, now adding autopsy tissue as a new source for morphometry applied to the development of peripheral nerve fibers in humans from 4 months before term to 104 years of age. A photograph of the 104-year-old lady from whom Jürgen Bohl got the sural nerve had just appeared in the local news paper because of her 104th birthday. Work on biopsies and muscle spindles continued. Work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) for 8 years.

It was shown by ocular micrometer measurements using the highest available light microscopic magnification at oil immersion that axons in all nerves studied reached their largest normal dimensions earlier, at about 3-5 years of age, whereas their myelin sheaths approached normal adult values not before puberty, i.e., at about 13-15 years of age. These results were important in respect to evaluating biopsies in children. A detailed study on *abnormal axon/myelin ratio*s in human nerves followed. The nerves from autopsy cases were in Mainz professionally selected and excised by Jürgen Bohl, a colleague from the time in Frankfurt/M., dedicated to careful preparation of peripheral nerves (Schröder et al. 1978).

Original cases described in Mainz

Amyelination: Congenital peripheral and central amyelination was detected in an 8 day-old, mature newborn who came to autopsy (Schröder und Bohl 1978). Approximately 40 years later in Aachen (Chapter E), JMS received another sural nerve biopsy with amyelination. Semithin sections of the whole sural nerve revealed a single small myelinated fiber only. This case was clinically diagnosed as a neurological variant of Waardenburg syndrome type 4 (i.e., PCWH = *p*eripheral demyelinating neuropathy, *c*entral leukodystrophy, *W*aardenberg syndrome and *H*irschsprung disease, OMIM #609136) caused by a novel heterozygous base exchange in *SOX10* (Parthey et al. 2012). In addition to the myelin deficiency, counts and calculation of the total number of non myelinated axons revealed a severe reduction of their number compared to unmyelinated axons in controls. Furthermore there was a considerable number of nonmyelinated axons that were larger than

normal unmyelinated ones. It was concluded that these were promyelin fibers that could not be myelinated because of the *SOX10* mutation.

Amyelination needs to be distinguished from *aplasia of myelinated fibers,* morphometrically evaluated and described in a sporadic 14-year-old girl (later in Aachen; Chapter F) (Schröder et al. 1993). Appropriate classification and molecular genetic studies are not yet available in this unique case which represents another characteristic developmental disorders of the peripheral nervous system.

Comparing developmental disturbances of nerve fibers, an unique *hypertrophic dysplasia of the perineurium* was described (Schröder et al. 1999; Thomas et al. 2000). The changes consisted of proliferated and abnormally arranged perineurial cells with protrusions extending into the endoneurium. Some cells showed innumerable indentations encircling collagen unlike those reported in any other perineurial cells. Extensive investigation in both cases failed to establish the cause.

Another type of severe alteration of perineurial cells was seen in the sural nerve from a patient with *'atypical' Cogan's syndrome*, a sporadic clinical entity with non-infectious interstitial keratitis and vestibuloauditary dysfunction and, as shown for the first time in this case, with a severe and unique type of peripheral neuropathy (Nolte et al. 2008). These cells were globular in shape and, unlike the dysplastic perineurial cells mentioned above, associated with endoneurial blood vessels. Some contained collagen fibrils and basal lamina material. The immunohistochemical reaction against EMA (epithelial membrane antigen) identified these cells as perineurial cells.

Further interesting changes of perineurial cells were already noted in Frankfurt a. M. (see Chapter D) after experimentally implanting *autologous, isologous, homologous, and heterologous nerve grafts* about 1 cm in length *heterotopically*, i. e., subcutaneously, under the dorsal skin of rats. These grafts were studied 1, 2, 3, and 4 weeks after surgery. An unusual sequence of changes was noted in the *heterolous implants*: After 1 week, the perineurium unlike in the other nerves appeared largely preserved. At 2 weeks invasion by inflammatory cells had begun. After 3 weeks the perineurium was severely thickened showing up to 20 cell layers. This was basically due to an increase of the number of perineurial cells and due to flattened inflammatory cells that had invaded the perineurium. The myelin breakdown was

delayed when compared to the regular time course of Wallerian degeneration seen in the *autologous* and *isologous* implants. The heterologous perineurium had obviously delayed myelin breakdown, functioning as some kind of immune barrier (Schröder 1972). At 4 weeks the perineurium was penetrated and the myelin breakdown had been initiated and progressed. The changes of the perineurium and the associated myelin breakdown was graded and related in a Table. Additional inflammatory reactions have also been illustrated light and electron microscopically. Because of lack of time, these results were only published in the proceedings of a pharmaceutical company at an international congress, not in a peer reviewed journal.

Experimental microembolic infarcts of skeletal muscles and peripheral nerves

Using the technique previously used to induce microembolic brain infarcts in cats (see above, Chapter B), and the technique for the perfusion fixation of peripheral nerves via the abdominal aorta as practised by Henry deF. Webster (see Chapter C), a student, Eberhard Lang, combined both methods. From the affected muscles in the lower leg he cut serial sections and produced 2- or 3-dimensional drawings relating emboli to infarcts. The Hostalen microspheres used had a diameter of about 30-37 µm and got stuck in terminal or preterminal blood vessels where they were easily identified as isolated or sometimes clustered emboli. The number of spheres injected into the abdominal aorta was crucial in respect to the size of infarcts. Only part of his art work from his *summa cum laude* thesis was reproduced in "Pathologie der Muskulatur" (Schröder 1982).

Pathology of skeletal muscle ("Pathologie der Muskulatur")

Routinely engaged in "Brain cutting", brain tumor diagnostics, teaching etc. JMS collected material for the first and representative myopathological book in German on "Pathologie der Muskulatur" (see above: Chapter C and below). Erich Kuhn who was already mentioned above, and some neurologists were the first ones to provide selective biopsies of muscles and peripheral nerves in Germany (e. g., Krücke et al. 1971: Thalidomid neuropathy).

In 1971 Wilhelm Doerr, general pathologist in Heidelberg, one of the editors of a series of books on "Spezielle pathologische Anatomie" (Special pathological Anatomy), asked JMS to write such a book. Erich Kuhn, myologist, Internal Medicine, also in Heidelberg known to JMS from the connection with Ray D. Adams, Boston, USA (see Chapter C), had recommended JMS as a possible author. Doerr had planed a compact book on skeletal muscles, joints, and bones ("Bewegungsapparat") for which several

authors should be engaged. However, JMS insisted on writing the book on skeletal muscle separately and as the single author. He knew from other multi-authored books that some co-authors were forced to wait up to e. g. 14 years finally quarrelling on court. He agreed and thereafter decently called him up every year until JMS delivered the whole volume to him as a heavy load in 1980. It got finally published as a monograph with 813 pages in 1982.

As indicated above the idea was to promote wider distribution of the book by thus far unpublished new fine structural results. Because of the unlimited time scale, collection of material and literature increased steadily Thus some of these results were preliminarily buried in this book or in proceedings of relatively small myology meetings, e. g., *"calcium deposits in the soleplate in tertiary hyperparathyreodism"* (Schröder, Krämer et al. 1980) which has not been described or illustrated elsewhere; or *"subsarcolemmal-segmental myofibrillolysis"* (Schröder 1982) which had meanwhile been published by others under the name of "cap myopathy" (Fidzianska et al. 1981).

Neuroferritinopathy: On the other hand, an obviously new disease which in this book (Schröder 1982) was named "Unclassified system disease with granular hyaline nuclear inclusions" ("Unklassifizierte Systemerkrankung mit granular-hyalinen Kerneinschlüssen") was in fact described and documented using 4 light micrographs and 2 electron micrographs before others described similar changes in "Neuroferritinopathy", or "Neuroferritinosis". This case was more extensively described as *"Granular nuclear inclusion body disease: Fine Structure of tibial muscle and sural nerve"* (Schröder et al. 1985) and later under the name of "Ferritinopathy" (Schröder 2005) after the underlying genetic defects had been identified (Curtis et al. 2001; Crompton et al. 2002).

It might be added that the editor of 'Muscle & Nerve' rejected reference to the first though less complete publication in "Pathologie der Muskulatur" (Schröder 1982). He seemingly wanted the new publication to be the first one on "granular nuclear inclusion body disease" as it was called at that time. In this original article histochemical iron positivity of the inclusions was also already described and documented in a table though not illustrated in an image because the number of 10 large light and electron microscopic figures had approached the editorial limit (Schröder et al. 1985).

Marinesco-Sjögren disease: Another example for the first description and illustration of what was considered at that time as another unique nuclear change in an apparently new disease was named "*Nucleodegenerative myopathy*" ("Nukleodegenerative Myopathie"; Schröder 1982, P. 272-276; Fig. 78 a-h). The

changes consisted of pleomorphic alterations, mainly separations of the outer lamina fibrosa of nuclei associated with membranous cytoplasmic bodies and other nuclear changes in muscle fibers although not in other cells. The changes turned out to be typical for Marinesco-Sjögren disease (Sewry et al. 1988). Later underlying mutations were found in *SIL1* by our and simultaneously another group (Anttonen et al. 2005; Senderek et al. 2005).

Writing the book was potentially an endless task basically collecting and arranging representative illustrations of own material as well as translating/modifying and dictating the original English text of others into the German language. The book was rigorously finished after receiving the offer for a full professorship in Aachen in 1980 (see Chapter E), nearly 10 years after the contract, somehow gluing all typewritten extracts together in a logical way at night while JMS was with his wife and both sons on holidays at Christmas time for skiing during daytime. The book basically introduced histochemistry and electron microscopy into the diagnostics of neuromuscular disorders in Germany at an international level.

Move from Mainz to Aachen, Germany

The gigantic new university hospital building in Aachen (total length: ca. 250 m) had not been completely finished in 1980 when JMS received the offer for a C4-Professorship at the well kown Rheinisch-Westfälische Technische Hochschule (RWTH) in Aachen. JMS delayed bargaining with the Dean and the administrators for more than a year. The family had just entered a new house (Fig. on page 30) built on a 951 m² piece of ground which had been offered by the university real estate foundation on lease ("Pacht") for '100 years' to JMS as a young professor. The area was located on a beautiful site above Mainz superviewing the river Rhine at its famous wine area, based on secularised country sites previously owned by the Church from which it was transferred to the university at the time following the Napoleonic revolution after 1789. To leave the new house was not an easy decision because it had been constructed according to individual plans and demands at that time. Also the climate and traffic connections were more appealing in Mainz than in Aachen. Finally the professional prospects were decisive: The position in Aachen was more attractive and the family followed in 1981 at a time when the two boys had just to go to school 6 months later.

Chapter F. C4-Professor at the Rheinisch-Westfälische Technische Hochschule (RWTH University) Aachen, and Head of the new Department of Neuropathology at the University Hospital: 1981-2004: *211 articles*

Team of the Department of Neuropathology in Aachen, Germany, in front of the giant new hospital building, 254 m in length, representing exceptional architecture in a green surrounding

After seven years in Frankfurt am Main and seven years in Mainz, JMS was offered a position as full (C4) professor and head of a new department of neuropathology at the Medical Faculty of the "Rheinisch-Westfälische Technische Hochschule (RWTH University) Aachen", Germany, in 1980. The Medical Faculty was created in 1966 at the RWTH which had been founded in 1881. But not before 1983 could the members of the clinical-theoretical institutes enter the building as an 'experimental group' to test the new hospital's function. The building became famous because of its architecture resulting finally in 'Denkmalschutz'(federal monument protection).

Work initiated in Boston (Chapter C), Frankfurt/Main (Chapter D), and Mainz (Chapter E) was continued in addition to evaluating autopsy and biopsy material of peripheral human nerves and skeletal muscles aside from routine work which in neuropathology may leave time for research. In addition moleculargenetic techniques were introduced into neuropathology using archival and fresh nerve and muscle biopsies as described subsequently.

(1) Experimental work
(2) Immunohistochemistry
(3) Morphometry
(4) Electronmicroscopy
(5) Molecular genetics

Ad 1. Experimental work

(a) Regenerating peripheral nerves in silicon tubes: Work on regenerating nerve fibers was now continued in rats using silicon tubes for comparing the effect of denervated muscle, fat, and nerve tissue as a target for axonal sprouts growing in a silicon tube after transection (Weis and Schröder 1989a). Perineurial cells or their pre-cursers were the first ones pioneering reinnervation of the distal stump (Schröder et al. 1993; Weis et al. 1994). At the *first step of reinnervation* up to 4 weeks after surgery, the central cord in the tubes showed similar cross sectional areas when connected distally to muscle, nerve, or fat tissue and evaluated planimetrically. Yet at a *second step* after 6 – 8 weeks tubes connected to fat tissue developed much smaller cross sectional areas (below 0.1 mm^2) than those connected to nerve and muscle tissue (cross sectional area up to 0.4 mm^2). It was concluded that nerve fibers making contact with adequate peripheral targets, i. e. nerve and muscle tissue, induced via unknown, presumably electrophysiological or biochemical retrograde effects, an increase of the number of outgrowing nerve fibers and better development in the connecting cord than fat tissue (Weis and Schröder 1989b). Fat tissue had obviously a negative retrograde effect clearly inhibiting regeneration to some extent.

The next step was to use this negative effect of fat tissue on regenerating peripheral nerves for inhibiting or reducing neuroma formation (Weis et al. 1998a). In fat, and in denervated, pre-atrophied muscle tissue growth of minifascicles occurred, mainly around blood vessels. After 24 weeks there was at least one limiting perineurial cell layer separating minifascicles from neighbouring fat cells. By contrast, nerve fibers could contact capillaries directly without an intervening perineurial cell. Nevertheless both, nerve fibers and capillaries were covered by a more or less complete and

separate basal lamina with an extracellular space between them. Otherwise a negative growth effect of fat tissue was difficult to prove.

Attempts to reinforce nerve fiber growth by adding nerve growth factors such as CNTF or recombinant variants, or IL6, resulted in prolonged survival of dorsal root ganglion cells *in vitro* (Simon et al. 1996) but application *in vivo* using some kind of regeneration chamber was not initiated because of the low chance to get experiments on animals allowed by the administrative authorities. Yet the adaptation of the earlier *organotypic tissue culture model* as previously used for investigating the direct effects of cadmium on neuronal cells ("Itai-Itai" disease; Tischner and Schröder 1972; see Chapter D) was successfully used for the growth factors mentioned and later for pharmacological studies on zidovudine (see below).

(b) Organotypic cultures. Co-cultivated spinal ganglia were used as before (Tischner and Schröder 1972), now together with muscle tissue, and anterior horns of the spinal cord to establish some kind of lower reflex arc *in vitro*. The idea behind were previous studies on muscle spindles in Frankfurt a. M. and Mainz (Schröder 1974). The connection of axonal sprouts worked out so impressively that Springer Company selected a representative phase micrograph of a well structured neuronal interconnection (Fig. 12d) from "Pathology of Peripheral Nerves. An Atlas of Structural and Molecular Changes" (Schröder 2001) to use it for its cover.

The effect of different doses of *zidovudine* on nuclei and mitochondria was evident *in vitro* showing focal defects of the nuclear membranes, dispersion of nuclear chromatin and mitochondrial swelling (Schröder et al. 1996). The changes were not as impressive as the lesions in a human muscle biopsy after high doses of zidovudine during 15 months for treating AIDS (Schröder et al. 1992): Adjacent nuclei were covered by a common nuclear membrane. Other nuclei were swollen, homogenized, or condensed; the heterochromatin was segregated from the nuclear membrane in other nuclei; the outer nuclear membrane was focally separated forming unusual blebs; and the perichromatin granules and nucleoli were conspicuously enlarged. Many mitochondria were also severely enlarged and abnormally structured. Similar nuclear abnormalities had not been reported in any other AIDS patient with or without treatment. Because of the high dosage of zidovudine and the structural alterations, the changes were attributed more likely to zidovudine than to AIDS.

(c) Muscle spindles: Another project followed over several years was focused on light microscopic, transmission electron microscopic (TEM), and scanning electron microscopic (SEM) studies on muscle spindles. For the first time three dimensional SEM aspects of different intrafusal motor and sensory endings were presented (Schröder et al. 1989; Dieler and Schröder 1990; Dieler et al. 1992). The moderate increase of the number of intrafusal muscle fibers following repeated de- and reinnervation as mentioned above, did not approach the number of 50 intrafusal muscle fibers as seen in human muscle spindles of cases with myotonic dystrophy (Dieler and Schröder 1990).

(d) Alcoholic neuropathy: The direct effect of different concentrations of ethanol on freely exposed sciatic nerves of rats was the last *in vivo* experiment of JMS. However the referee of the local governmental commission for reviewing grants in Düsseldorf, NRW, considered such a study as 'too mechanical' (sic!) and rejected the proposal. The social importance and the technical competence to perform such an experimental series had obviously not strongly enough been elaborated. Nevertheless a few images of ethanol induced segmental lesions could be published (Schröder 1999). As mentioned already, political decisions largely prohibited experimental studies in living animals. Therefore studies on nerve and muscle diseases were subsequently focused on molecular genetic aspects (see below).

Ad 2. Immunohistochemistry

There are nearly innumerable immunopathological markers to identify and differentiate inflammatory, 'rheumatic', paraneoplastic, and other diseases using semithin or ultrathin, plastic embedded nerve biopsies with higher resolution power than available through the widely used paraffin or cryostat sections. Two studies revealed especially interesting news:

(a) This was the immuno-electronmicroscopic identification of amyloid fibrils in vacuoles of endoneurial macrophages in amyloid neuropathy (Sommer and Schröder 1989). This observation is of more general interest since it appears to be representative for futile attempts of macrophages to remove amyloid and other protein aggregates from the tissue. This may concern other diseases as well. At a Peripheral Nerve Association (PNA) meeting in Halifax Peter J. Dyck helped to summarize and to explain the results while JMS as the presenter had difficulties to

raise interest in the audience late at night after a heavy Canadian meal with wine and other drinks. Important results have to be propagated to get recognition!
(b) Cooperation with Berkiels et al. resulted in the first publication of molecular defects in periaxin neuropathy and immunohistochemical documentation of circumscribed periaxin deficiency in teased peripheral nerve fibers (Takashima et al. 2002).

Ad 3. Morphometry

Previously it was shown that during development of sural, femoral, radial, facial and trochlear nerves, axons reach their adult values at about 5 years, whereas myelin sheaths approach their adult values not before about 15 years of age (Schröder et al. 1978; 1988). At the paranode axons are about half as thick as at the internode (Bertram and Schröder, 1993; Schröder 1996). The ratio between internodal and paranodal diameters remains relatively stabile with an average value of about 1.8 to 2.0 (range: 1.6 to 2.5). The length of the attachment zone of myelin lamellae to the paranodal axon reaches its upper limit of 3-6 µm already at about 2 months of age. Surprisingly it does not increase further in proportion to the increasing number of myelin lamellae; this results in myelin lamellae ending not-attached to the axon in the paranode loosing or not gaining transverse bands. Freely ending myelin lamellae result in characteristically distented myelin sheaths.

Optic-electronic determination of myelin density was a good and easy time sparing method for histopathological determination of the severity of a neuropathy (with the exception of neuropathies with selective myelin loss such as amyelination or absence of large myelinated fibers) (see Chapter E). The normal lower level was approximately 20% of the endoneurial area; myelin area percentage values below this level were considered abnormal (Schröder and Seiffert 1972). The KS300 system of Kontron (Eching/Munich, Germany) permitted printing of three-dimensional columnar diagrams which represented myelin thickness (abscissa = x-axis), axonal diameter (z-axis), and number of nerve fibers per column (ordinate = y-axis) (Schröder 1998; Senderek et al. 1998). This was of help for illustrating the severity of a neuropathy in general. (Surprisingly, this method has not been used elsewhere.)

A common and prominent fine structural change initiating paranodal or segmental intermodal demyelination appears to be retraction of myelin lamellae from the axon

with occasional myelin loops underlying others in the paranode ('axo-glial dysjunction', a definition that is in use despite Schwann cells are not 'glial cells'). Incorporation of axoplasma into the paranodal region is equally impressive, especially when these axonal outpouchings are filled with various axonal components such as mitochondria, lysosomes, vacuoles with and without glycogen granules, and membranous cytoplasmic bodies. In a review (Schröder 1996) normal and abnormal changes at the node of Ranvier were documented in two developmental diagrams, 7 electron micrographs and a differential diagnostic list summarizing the most important developmental, pathological, and artificial changes at the node of Ranvier. This list was later extended (Schröder and Weis 2014).

Ad 4. Electron microscopy

Nearly all 330 scientific publications in which JMS has been involved are based on 'ultrastructural' (= electron microscopic) studies of neuropathological, mainly neuromuscular changes, especially of peripheral nerves. There was a short discussion in one of the first sessions of the Medical Faculty which JMS attended that electron microscopy ought to be available for every faculty member including orthopaedists and gynaecologists. The question, however, was who could operate the sophisticated instruments and would effectively use these sensitive techniques. Finally specimens submitted by neurological, neurosurgical, neuropediatric and certain other clinics should be taken care of by the department of neuropathology, others by the department of pathology.

Ad 5. Introduction of molecular genetic methods into neuropathology using archival paraffin embedded tissue

From Anita Harding, the first female professor at the famous Neurological Institute, Queen's Square, London, GB, at that time wife of Professor Peter Kynast Thomas ("PK"), Department of Neurology at the Royal Free Hospital in London, GB, one of the students of JMS - Ruth Thiex – learned the polymerase technique and the technique of sequencing DNA segments for identifying genomic mutations. Thiex adapted these techniques to paraffin embedded tissue. Anita Harding stated competently from her experience that these techniques were easier to handle than electron microscopy.

CMTX. Other highly motivated students (Jan Senderek, Bergmann and Stefan Züchner) were allowed to use the sequencer in the Microbiology Department of the Hospital in 'Aken' (Aachen). From Vincent Timmerman in Antwerpen JMS received probes with proven mutations from a case of X-linked peripheral neuropathy (CMTX) as pathologic controls for confirming the mutations identified in archival, paraffin embedded peripheral nerve tissue. The mutations were new ones and could be published in combination with corresponding light and electron micrographs (Senderek et al. 1998, 1999). The correlation of molecular genetic data with fine structural observations was at that time of general interest to editors of scientific journals and their reviewers. Cytoplasmic dilatations of adaxonal myelin lamellae in CMT X were associated with irregularities of transverse bands in the 'axoglial' conjunction zone. Nerve biopsy findings from a mother and her two boys were later illustrated by optic-electronic videoanalysis in three-dimensional diagrams (cf. Schröder and Senderek 2014).

Incidental combination with *Becker's muscular dystrophy, also X-linked*, was documented in one of the cases ('double trouble'). Yet there was no evident molecular genetic link between the two disorders (Bergmann et al. 2000). In another family additional X-*linked red-green colour blindness* was recorded. But there was also no close linkage between these two entities. An association with tremor or *Roussy-Lévy syndrome* was interpreted as non specific because tremor was also observed in other neuropathies. Mutation in the promoter region of the Connexin (*Cx*) gene was found to be clinically insignificant (Bergmann et al. 2001).

CMT1A and HNPP. Extraction of DNA from paraffin-embedded peripheral nerves was successfully performed also in a series of cases with PMP22 multiplication causing CMT1A (14 cases molecular genetically identified using archival, paraffin-embedded material), and a similar number of cases with a neuropathy with liability to pressure palsy (HNPP) due to deficiency of PMP22. These cases were compared to 133 control nerves from the archive (Thiex and Schröder 1998) and revealed several interesting observations in addition to molecular genetically confirming the clinically suspected diagnosis:

(1) *Comorbidity* of CMT1A with *diabetes mellitus* resulted in severe cases in nearly complete loss of myelinated fibers (e.g., in case 8: 0.08 % of the endoneurial area; normal value: 20-30%); in addition there was absence of onion bulb formations

which otherwise are so typical for CMT1A. This was confirmed in a later study using a new quantitative PCR technique for reliable gene dose determination of highly degraded DNA from up to 12-year-old sural nerve biopsy samples by 'direct-double-differential PCR' (Beckmann and Schröder 2000).

(2) *Exposure to toxins* aggravated CMT and had similar effects as diabetes mellitus in respect to the severity of a neuropathy (Senderek et al. 1999).

(3) *Optic-electronic evaluation* of the area of myelin sheaths per endoneurial area on semithin cross sections using the highest light microscopic magnification with the apochromatic lenses of Zeiss, Oberkochen, Germany, and the program VIPER (video image processing, evaluation, and recording system of Gesotec, Darmstadt, Germany) (Schröder 1998), revealed a striking difference of the severity of the neuropathy caused by *PMP22* duplication versus *PMP22* deletion: In a comparable adult age group it was more severe in CMT1A (due to a duplication) than in HNPP (due to a deletion). Furthermore, a single tomaculous fiber could already indicate HNPP as confirmed by a deletion of *PMP22*. On the other hand, some tomacula could occur despite duplication of *PMP22* indicating CMT1A (Thiex and Schröder 1998).

New DFG and KlausTschira grant

With the background of the large number ca. 8 000 nerve biopsies and 12 000 muscle biopsies documented in a relational data bank and on the basis of publications JMS applied for a grant from the German Research Foundation (Deutsche Forschungsgesellschaft; DFG). But not before two further years of practicing and publishing papers related to the subject did he succeed in the year 2000. The grant allowed to hire a molecular genetically experienced postgraduate fellow as well as to acquire laboratory equipment to use the archive for studying tissue and families. In fact several new mutations and genes were identified causing peripheral neuropathies and myopathies.

A KlausTschira Grant provided another position for a scientific assistant to analyse Guillain Barré cases from the data bank for differentiating HLDR subtypes causing a disposition for the disease and for finally treating this immunogenetic disorder. Lack of time did not allow to complete the study and to publish results before JMS retired.

The following articles are based on this advantage and are listed in chronological order of appearance

The name or substrate of a disease is listed in the following articles in italics as the second one; the first author with the year of appearance of the article as the third one. Articles identifying genes for the first time as the cause of the disease are marked by an asterisk. Keywords or highlights are briefly summarized behind these data:

CMT1A and HNPP, *PM22* (references see above)

CMT1B, *P0 (MPZ)* (Senderek et al. 2000): Hotspot on Thr124 Met in the *P0* gene versus founder effect in a Belgian family. Progressive loss of nerve fibers within 4 years, documented because of clinically controversial diagnoses by two subsequent sural nerve biopsies. Truncating mutations do not necessarily cause a severe phenotype (Senderek et al. 2001).

CMT X, *GJB1, Cx32* (see above)

IR-CMT, *GDAP1* (Senderek et al. 2003): axonal as well as demyelinating (intermediate recessive) lesions with no significant mitochondrial alterations in two families. *GDAP1* is obviously vital for both, axonal integrity and Schwann cell properties.

Tangier disease, *ABC1* (Züchner 2003): In a pseudosyringomyelic form of the disease severe *endoneurial fibrosis* was associated with loss not only of myelinated and unmyelinated axons but also of Schwann cells which is quite unusual. Very few myelinated fibers were preserved. Endoneurial macrophages were rare but characteristically loaded with lipid droplets that were not membrane-bound.

CMT4C*, *KIAA1985* (Senderek et al. 2003): Newly identified homozygous or compound heterozygous mutations of this novel gene are the cause of a congenital demyelinating neuropathy with unusual, obviously characteristic slim Schwann cell processes. These Schwann cell processes connect isolated unmyelinated axons with each other. The mutations of the gene are responsible for alterations in a protein of a new protein family of unknown functions, i.e., *KIAA1985.*

CMT1F, *NEFL* (Züchner et al. 2004): A partly demyelinating, partly axonal, i.e. 'intermediate' form of a dominantly inherited neuropathy: for the first time illustrated and genetically identified in this type of neuropathy.

CMT2A*, *Mfn2* (Züchner et. al. 2004): Missense mutations in the mitochondrial fusion protein mitofusin 2 (MFN2).

This most successful article of all 330 listed articles in which JMS was actively engaged (cf. list of references) came out in 2004, at the end of his official carrier, 1.5 years after official prolongation of his position following obligatory retirement in the year 2002. Thirteen years later in February 2017 this 'Brief Communication' has been quoted according to *Scopus*, the data bank of Elsevier, altogether 814 times (on February 3, 2017). According to Google Scholar the number of citations was even higher: 1119 (July 12, 2017) because quotations in articles or paragraphs of books or proceedings are included.

The first of altogether 24 authors in this basically 3-page paper is Stephan Züchner who was a student of JMS and who had recently finished his doctoral thesis. Based on his doctoral thesis and additional molecular genetic work combined with light and electron microscopy studies in JMS' department, he received a grant of the DFG for a research fellowship in Durham, Atlanta, USA. Together with a group of European co-workers a paper was published shortly before the Nature Genetics paper appeared presenting negative findings in a Turkish family (Bissar-Tadmori et al. 2004) stressing that the cause of European CMT2A cases was not *KIF1B*, as published by a Japanese group (Zhao et al. 2001). A recent study of Chen et al. (2003) revealed that mitofusin 2 was important for fusion and fission of mitochondria in tissue cultures resulting in severe alterations when mutated. Application of this knowledge to human CMT 2A cases resulted in the detection of mutations of *mitofusin 2* as the cause of CMT 2A. The paper came out from the Department of Neuropathology, University Hospital, RWTH Aachen, Germany, from which JMS was going to retire, and the Center of Human Genetics, Duke University Medical Center, Durham, North Carolina, USA. JMS was the last before the last author (Jeffery M. Vance) both of whom were mentioned as having "contributed equally to this study".

It should be added that a number of preceding studies of JMS had already been focused more or less selectively on mitochondrial alterations or diseases caused by mitochondrial DNA mutations whereas nuclear genes causing mitochondrial defects were less frequent (Peiffer et al. 1988; Sommer and Schröder 1989; Schröder and Sommer 1991; Schröder 1992; Schröder et al. 1995; Molnar et al. 1995; Molnar et al. 1996; Schröder et al. 1996a, b, c; Zanssen et al. 1997, 1998; Molnar and

Schröder 1997; Schröder 1997; Bank et al. 2000). Verhoeven et al. (2005) described for the first time the fine structure of mitochondrial changes in cases with identified *Mfn2* mutations.

The following articles were supplied with light and electron micrographs while the molecular genetic analyses were performed in other laboratories

Optico-cochleo-dentate degeneration *due to peroxisomal D-bifunctional protein deficiency* (Schröder et al. 2004): Autopsy case with severe peripheral neuropathy which has not been investigated in previous cases. There were characteristic cytoplasmic inclusions mainly in perivascular macrophages and astrocytes showing a bilaminar structure whereas trilaminar structures, typically seen in adrenoleukodystrophy, and multilaminar structures were less frequently observed.

Duchenne muscular dystrophy*, Dystrophin mutations* (Wang et al. 1998): The frequency of revertant muscle fibers in muscle biopsies of cases with molecular genetically defined DMD were obviously not correlated to the severity of the disease.

Burning feet syndrome, distinct autosomal dominant genetic entity (Stögbauer et al. 1999): neuropathy predominantly affecting small, unmyelinated fibers.

Infantile mitochondrial myopathy *and* neuropathy, *Leu[UUR]* (Zanssen et al. 1997): *multiple mitochondrial tRNA mutations.*

CMT4F (Periaxin neuropathy), *PRX* (Takashima et al. 2002): See above paragraph on the only immunohistochemically verified case with partial L-periaxin deficiency. The first step of the pathogenesis of this demyelinating type of neuropathy appears to be focal separation of myelin loops from the axon with deficiency of paranodal transverse bands.

Rippling muscle disease, *Caveolin-3* (Kubisch et al. 2003): Nearly complete absence of caveolae in the sarcolemma.

Actin myopathy, *ACTA1* (Schröder et al. 2004): Actin deposits in the sarcoplam *and* nuclei of muscle fibers.

The next group of papers was written without final molecular genetic clarification

HMSNL, 8q24 (Baethmann et al. 1998): Unusual double-layered, often semicircular or curvilinear axonal inclusions with serum vitamin E in normal limits. (The underlying mutation in *NDRG1* was detected later in other cases.)

Polyneuropathy with osmiophilic double membrane-bound 30-600 nm cytoplasmic inclusions in Schwann cells (POMCIS), no evidence of inheritance (Schröder et al. 1999): chronic, slowly progressive demyelinating neuropathy evaluated morphometrically, with unique ultrastructural features as mentioned in the title.

Dysplastic perineurium, possibly recessive inheritance, no gene detected (see above; Schröder et al. 1999): slowly progressive neuropathy. The changes were thought to represent an unusual but non-specific response to a peripheral neuropathy (Thomas et al. 2000) (cf. Chapter E).

HMSN-ADM, rare developmental type of neuropathy (cf. Chapter E) the genetic defect of which has not been identified (Müller et al. 2000): Unique morphometric analysis of the absence of large myelinated fibers with deficiency of large neurons in the spinal ganglia and anterior horns of the spinal cord, associated with deafness, mental retardation, and epilepsy. Degenerating endoneurial cells (cf. Grehl and Schröder 1991) are more impressive than those in other neuropathies.

Myotonic dystrophy (MD) combined with neuropathy (Wang and Schröder 2000): Archival biopsies from the time when genetic data were not available. Minor to mayor reduction of the percentage of the myelin area per endoneurial area in all 17 patients with MD evaluated morphometrically. In some cases the myelin sheaths appeared to be unusually thick. This is a representative number of MD cases biopsied for diagnostic purposes by a combined muscle and nerve biopsy.

Myopathy with novel tubular aggregates. Familial disease; gene not yet detected (Müller et al. 2001): Tubules 30-200 nm thick in diameter which included 1-21 tubulofilamentous structures, 14-18 nm in diameter. Type II fibers were affected showing multiple subsarcolemmal basophilic aggregates staining red with modified trichrome and intensively blue with the NADH-tetrazolium reductase. The aggregates

were not reactive for SHD or COX. Yet oxidative phosphorylation was defective (Vielhaber et al. 2001). The myopathy is of a benign, slowly progressive type with late onset, muscle pain, cramps, and stiffness.

Endoneurial and epineurial blood vessels: diagnostic impact. The number of epineurial vessels may increase from about 50 (34 – 76) in normal sural nerves to 196 in panarteritis nodosa with inflammatory occlusion of the main artery of the sural nerve (Schütz et al. 1997). No direct correlation was noted between the number of endoneurial and epineurial blood vessels (Mawrin et al. 2001).

In general, blood vessels in nerve biopsies are of high value for the diagnosis of the general state of small blood vessels in the body. It was shown for the first time that **CADASIL** can be diagnosed by specific granular osmiophilic deposits on the surface of smooth muscle cells of blood vessels in the sural nerve (Schröder et al. 1995). Ten years later, mutations in *Notch 3* causing the disease as shown by others were also available for the diagnosis in local cases (Schröder et al. 2005).

Pathognostic granular intranuclear inclusion bodies in **ferritinopathy** were shown for the first time in a muscle and nerve biopsy (Schröder et al. 1985; 2005). No cerebral biopsies were needed anymore for both of these meanwhile genetically characterized diseases: CADASIL and ferritinopathy.

Certain **lipidoses** and **ceroidlipidoses** can also be identified by characteristic inclusions in endothelial cells of blood vessels and other cells of peripheral nerves (cf. Schröder 2001).

Inflammatory polyradiculoneuropathy with spinal cord involvement and letal outcome after **hepatitis B vaccination**, was noted in a sporadic case (Sindern et al. 2001): autopsy with immunohistochemical identification of inflammatory cells. The inflammatory infiltrates in the spinal cord resembled those in patients with Guillain-Barré syndrome (Müller et al. 2003).

Peripheral neuropathy in neurofibromatosis type 2, inactivation of *Merlin* suggested (Sperfeld et al. 2002): Unusually large onion bulb like clusters of Schwann cells dominate the biopsy findings in one sural nerve of 15 clinically studied patients. Some cells in these *tumourlets* showed extraordinary large, irregularly indented nuclei, and multiple cytoplasmic indentations or vacuoles, partly covered by a basal lamina, not seen in ordinary onion bulbs.

AT-EAN in newborn Lewis rats, increased Ia expression (Pilartz et al. 2002): Inflammatory infiltrates and mast cell activation with only minor nerve fiber degeneration and no demyelination.

Further articles are discussed in Chapter G following retirement in the year 2004.

Chapter G. Emeritus since July 2004-2017: 30 *articles*

'Flower power' in the entrance of the private home at the occasion of the official retirement in 2004

Despite formal retirement at the end of winter semester February 2002, the academic duties and facilities of JMS were exceptionally prolonged by both, the Dean of the Medical Faculty and the Rector of the RWTH University in Aachen. This was repeated three times for 6 months each, with official ending after altogether 1.5 years, June 2004 (Fig. on page 50). Thereafter the successor of JMS, Joachim Weis, took over providing JMS with a room called "Denkzelle", think cell, equipped with computational power for further activities, mainly participation in publications without diagnostic activity. His role in publications since this time was adding cases from the **data bank** that he had initiated in 1966 and promoted until retirement. This data bank started as a simple digital one which during the years became a more sophisticated relational one with 24 items per case. At his retirement it comprised more than 8 000 nerve and 12 000 muscle biopsies in addition to neurosurgical and autopsy cases, the number of which had increased steadily and continues to increase since then. His role was voluntary cooperation with students or younger colleagues to improve manuscripts for publication, eventually providing frozen or embedded tissue, semithin sections, electron micrographs, and some clinical data from the data bank.

To keep in contact with the German Society of Neuropathology and Neuroanatomy (DGNN) after retirement, the **"Theodor Schwann Preis"** (Theodor Schwann Award) was inaugurated and equipped with a basic start capital of 5000 EURO by JMS to be presented to young neuropathologists in portions of 1000 EURO during the following annual meetings of the society. Small personal donations were added to the

basic amount. Thereafter the society was supposed to take over the responsibility. This was settled in a new formal contract as indicated on the Society's homepage.

The following articles were selected because they are correlating specific clinical descriptions with new electron microscopic or new molecular genetic data appearing **after retirement** of JMS (genes in *italics)*:

Limb girdle muscular dystrophy, *Calpain 3* (Jenne et al. 2005)
Marinesco-Sjögren syndrome, *SIL1** (Senderek et al. 2005; cf. Chapter E: Schröder et al. 1982; Roos et al. 2014)
((**Ferritinopathy**, *FLP* (Schröder 2005)) (cf. Chapter E)
CMT2A, *MFN2* (Verhoeven et al. 2006)
Cold-aggravated myotonia, *SCNA4* (Schoser et al. 2007)
Cogan's syndrome, Perineurial cells filled with collagen (Nolte et al. 2008a)
Congenital type IV glycogenosis, *GBE1* (Nolte et al. 2008b)
((**'Necklace fibers'** resembling trilaminar fibers, *MTM1*)) (Schröder 2009))
Botulinum toxin injected into a muscle of normal human controls (A.S. Schroeder et al. 2009)
CMT4C, *KIAA1985* (Weis et al. 2010)
OPMD, *PABPN1* (Schröder et al. 2011)
Amyelination, *SOX 10* (Parthey et al. 2012) (cf. Chapter E)
Transthyretin, *TTR* (Dohrn et al. 2013)
Neurofibromatosis, *Merlin Isoform 2* (Schulz et al. 2013)
Myofibrillar myopathy, *BAG3* (Semmler et al. 2014)
Cap myopathy, *TPM3* (Schreckenbach et al. 2014) (cf. Chapter E)

Recent reviews and articles in books

The 4th edition of the standard teaching book of *Neuropathology* in German appeared in 2012 (Editors: W. Paulus and J. M. Schröder). The chapters covering the pathology of nerves and muscle therein were written by JMS.

A monograph on "Peripheral Nerve Disorders. Pathology and Genetics" edited by J. M. Vallat and J. Weis (2014) includes further revue articles on peripheral nerves in part written by JMS.

A more recent review article "Towards a functional pathology of hereditary neuropathies" appeared in Acta Neuropathologica (J. Weis et al. 2016: 493-515) and was co-authored by several colleagues.

Number of JMS' publications and articles quoting his publications and 'Citation index' by the year 2017; Wikipedia

JMS' list of publications includes 330 original papers and reviews as well as articles in books and proceedings (cf. *PubMed*; and *www.ukaachen.de*/Kliniken & Institute/Institute mit Aufgaben in der Krankenversorgung//Institut für Neuropathologie/Institut/ Team/Alumni/ Univ.-Prof. Dr. J.M. Schröder/Lebenslauf, Publikationen). A computational documentation by *Google Scholar* listing the number of papers and chapters citing articles of JMS comprises on March 17, 2017 altogether 7022 quotations (h-index: 42; i10-index: 151). An appealing aspect of such a rigorous documentation is that the values do not decrease but increase with age.

Additional personal data of JMS are included in *Wikipedia's article on "Johann Michael Schröder"*.

Epilogue

'Farewell' at further retirement in 2014

Looking back at JMS' 80 years live balance, thereof 50 years specialised in neuropathology - starting with the first publication in 1964, and slowly ending in 2014 at official retirement - it looks like a uniquely linear, continuous progress. Nevertheless there were backlashes although compensable ones. An autoaggressive disease originated during military service and at the time of early disillusions. Lesions were moderately overcome by substitutions such as worn by his great ideal, Theodor Schwann (cf. the Fig. on page 78 in W. Haymakers and F. Schiller: "Founders of Neurology", sec. ed., 1970). Anyway, it did not matter when working in a laboratory or in an office with rather rare life contact to people including patients. Nevertheless he introduced muscle and nerve biopsy technics into German neuropathology to increase its clinical visibility and to overcome the autopsy gap. The basic aims of the intended carrier had been achieved, and impairments did not originate before retirement: central, peripheral, ocular, and autonomous symptoms of an organ system, which was subject of his basic interest. Thus he watched with professional awareness what was going on alongside presumptive aggregation of proteins, possibly synucleins, while ageing. Avoiding philosophy, he admires and adores the incredibly differentiated, morphologically accessible molecular and atomic world that continues to make up life and 'dead' material, the basic components which are still not completely understood to explain at least such a common phenomenon as memory. Nevertheless he continues to use his memory as well and as long as possible.