Abstracts
Joint Meeting of the German and Scandinavian Neuropathologists
Turku, Finland, June 3-4, 1983

Intracellular calcium and freeze fracture plasmalemmal changes of skeletal muscle cells in Duchenne muscular dystrophy (DMD).
The evolution of muscle cell necrosis in early stages and in male fetuses at risk

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In Duchenne muscular dystrophy (DMD), morphological abnormalities in freeze fractured plasma membranes of skeletal muscle cells (decrease of intramembraneous particles and orthogonal particle arrays) led to the hypothesis that a defect of the plasmalemma is correlated with a pathological influx of calcium into non-necrotic muscle cells in early stages of the disease. This is shown light microscopically in 40 Duchenne patients by Morin- and Alizarin-red-stains and electron microscopically by the KPA (potassium pyroantimonate)-calcium precipitation method. KPA-calcium precipitation is observed mainly in the mitochondria, in the nuclei, moderately between the myofibrils and subsarcolemmally (delta regions). Using atomic absorption spectrometry (Dr. Frey, Physiologisches Institut, Universität Freiburg) we were able to confirm these histopathological results. There is a highly significant increase in muscular calcium accompanied by a reciprocal decrease in magnesium: the ratio Mg\(^{++}\)/Ca\(^{++}\) decreases from 12.85 (healthy control muscle) to 2.16 (DMD). In contrast to DMD muscle (mean % of Ca\(^{++}\)-positive fibers: 7%) cellular calcium accumulation is rare in autosomal recessive and Becker-Kiener muscular dystrophies or other myopathies, and the plasma membrane structure in freeze fracture preparation is also significantly different from DMD. In 4 of 8 investigated male fetuses at risk for DMD, similar results could be obtained with regard to the calcium accumulation light and electron microscopically, as in affected DMD patients. In freeze fracture a high % of plasma membranes of these positive fetuses show a decrease of intramembraneous particles.

The results in early stages of DMD and in fetuses at risk for DMD support the hypothesis of a basic abnormality of the plasmalemma correlated with a pathological influx of calcium into the muscle cell together with a depletion of muscle magnesium. This might account for a mitochondrial decompensation and various metabolic disturbances, finally leading to cell necrosis. Calcium antagonists may have therapeutic value in DMD.
Experimental mitochondriopathy by electric stimulation of skeletal muscle in rats

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Abnormal mitochondria play an important rôle in very different kinds of muscle disease, but little is known about the morphogenesis of the mitochondrial alterations. As shown in biochemical studies, endurance exercise and chronic stimulation provoke, among other things, a greatly increased mitochondrial volume fraction and an increase of most mitochondrial enzymes. In the present study, the early morphological response of skeletal muscle mitochondria to electrically-induced short- and medium-term activity was studied.

The experiments were performed on anesthetized rats. The triceps surae muscle was electrically stimulated with 5 V for 20 μs with a frequency of 2 Hz. The duration of stimulation was 2, 5, 15, 30 or 60 min. By means of enzyme-histochemistry, an increase of oxidative activity and of RNA was demonstrated in many fibres. This corresponded to an increase in the number and size of muscle mitochondria. These alterations appeared especially within type I-fibres; morphometric investigations were therefore performed in red soleus muscle, measuring the mitochondrial mean diameter and the cut surfaces of the largest subsarcolemmal mitochondrial accumulations in 20 different fibres of each animal. After only 2 min of stimulation, the cut surfaces of the mitochondrial accumulations were already almost twice as large as in control animals and growing further with the duration of stimulation. The mean diameters of mitochondria grow within the first 15 min, then decrease again suggesting a division of giant mitochondria. All morphometric results were statistically controlled. In conclusion, mitochondria react to electrical stimulation: (1) after short term stimulation; (2) with increase in number and size; (3) obviously due to an energy deficiency in an unspecific attempt of compensation.

Enzymhistochemistry in normal pelvic sphincters

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Striated muscle from the urethral sphincter and the cutaneous and superficial part of the anal sphincter obtained during autopsies was studied. 7 cases were examined, 2 women and 5 men. The age range was 19-48 years, and 5 of them were under 30 years old. The interval between death and autopsy was from 16-72 h.

The classification of the individual fibers was obtained using the ATP-ase and the DPNH stains. Measurements of the lesser diameter were performed on type 1 and type 2 fibers.

Type 1 as well as type 2 fibers were present in the urethral and anal spinchters. In the urethral sphincter, about 85% of the fibers were type 1. The mean diameter was about 15 μm. The type 2 mean diameter was about 27 μm. 86% of the fibers of the subcutaneous anal sphincter were type 1. The mean diameter of type 1 was 15.6 μm and type 2, 32.8. In the superficial anal sphincter, the type 1 fibers constituted 83% of the fibers. The mean diameters of type 1 and type 2 were 16.6 and 32.2 μm, respectively.
Dietary deficiency of vitamin E, or alphatocopherol, and its homologues may be associated with disorders of the vertebrate reproductive system, skeletal muscle, nervous system, cardiovascular system, hematopoietic tissue and liver. Morphologic changes in skeletal muscle include segmental necrosis, phagocytosis, atrophy and regeneration of skeletal muscle fibers. In this report, we would like to focus on the sequence of the lesions in muscle fibers and on the distribution of procion yellow in muscle tissue.

45 weanling rats were fed a vitamin-E-deficient diet containing 6% linoleic acid, 2% stripped corn oil, and 2% glucose. 40 rats served as controls and were given a vitamin E supplement of 200 mg/day. The animals were sacrificed after 4, 8 and 12 weeks. Before sacrificing, we injected 1% procion yellow intraperitoneally 3 times. Biopsies of the quadriceps muscle were performed on both sides: one side was used for electron microscopy; the other for fluorescence microscopic studies. Serial frozen sections were cut, fixed and mounted unstained, or for enhanced fluorescence, frozen, dried cryostat sections were prepared.

In the control animals, the yellow fluorescence of procion yellow was confined to the connective tissue of normal muscles. In the first group (4 weeks), procion yellow could be detected only very rarely in isolated necrotic muscle fibers. Necrotizing myopathy was well developed after 8 weeks of the dietary regimen. There was a bright yellow fluorescence of procion in necrotic fibers of various stages. In addition, there was an increase in the number of muscle cells in the second group which revealed a globular green autofluorescence. In the third group (12 weeks), there was a further increase in the number of procion-yellow-stained necrotic fibers, reflecting the progression of the disease.

Under the electron microscope, the cytoplasmic reticulum in some areas appeared as a dilated tube-like network with irregularly arranged varicosities. In intact muscle fibers and in regenerated fibers, no defects of the cell membrane were detectable.

These results indicate that fiber necrosis develops concurrently with membrane defects rather than via prior latent membrane injuries.

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* This work was supported by a Feodor-Lynen Fellowship from the Alexander von Humboldt Foundation, Bonn.
Mixed nemaline-core myopathy with adult-onset
A new muscle disorder?

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Nemaline myopathy, central core disease, and minicore disease represent distinct myofibrillar disorders within the spectrum of congenital myopathies. Genetically, they apparently follow dominant traits, but sporadic cases also occur. Myopathies with a mixed pattern of nemaline rods, central cores, and minicores have been shown in rare patients with congenital or infantile neuromuscular disorders. We report the first sporadic case of adult onset in a 75-year-old female patient presenting with chronic progressive muscle weakness and wasting since the age of 60. Muscle biopsy revealed the coexistence of myofibrillar changes associated with atrophy of type 1 and type 2 fibers. Mixed nemaline-core myopathy will be discussed as a new variant of adult onset myopathies.

Nerve fiber lesions and the consequences interpreted by enzyme histochemistry

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Enzyme histochemistry, which selectively stains mitochondria, lysosomes, or membranes, reveals alterations produced by nerve fiber damage which are invisible with normal light microscopy. After nerve injury, acid phosphates accumulate quickly in the proximal and distal nerve stumps, while the rest of the axon remains free of acid phosphatase activity. While succinic dehydrogenase activity does not increase (mouse) or even decreases (rat) in the cell body, the activity of other mitochondrial dehydrogenases increases, suggesting a heterogeneity of mitochondria in the neuron. Moreover, the appearance of succinic dehydrogenase activity near the lesion suggests a local activation of axoplasm.

The observation that nerve fiber damage rapidly produces local reactions which may be independent of the cell body reaction may have wider relevance for the understanding of the axonal transport of organelles (vide Thomas: Veröff. Morph. Path. Heft 80, Fischer, Stuttgart u. Jena 1969; Hdb. d. Histochemie VII,5, Fischer Stuttgart 1977).
Experimental thalidomide neuropathy: the morphological correlate of reduced conduction velocity

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Thalidomide neuropathy has been extensively studied in man and experimental animals. Recently, sensory conduction velocity was shown to be reduced by approximately 11–15% after long-term application of thalidomide to New Zealand white rabbits. Morphological studies of experimental thalidomide neuropathy, however, have thus far failed to show significant structural changes.

The present investigation was performed on sural nerves of 10 female New Zealand white rabbits receiving thalidomide (100 mg/kg/die p.o.) for a period of 30 weeks. Rabbits of the same strain and equal sex, weight and number served as controls. Very few nerve fibers were seen undergoing Wallerian degeneration in either group, experimental animals or controls. Morphometry, however, revealed a slight though statistically significant difference of the mean myelin thickness of sural nerve fibers in the thalidomide group of rabbits as compared to controls. There were more fibers with thin myelin sheaths. The mean myelin thickness of the largest nerve fibers was also significantly smaller than in the control group. On the other hand, axonal diameters and internodal distances of the myelin sheaths were not significantly altered. The reduction of myelin thickness to some extent might be related to the reduced conduction velocity as well as to the reduced weight gain seen in the experimental animals for the period studied.

Ultrastructural and morphometric findings in sural nerves of children with autosomal recessive/sporadic hereditary motor and sensory neuropathy type I

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10 autosomal recessive/sporadic cases of hereditary motor and sensory neuropathy type I (HMSN I), 9 of which originated from the northern part of Sweden, were included in the study. Sural nerve biopsies revealed varying degrees of onion bulb formation. In 8 of the cases, the onion bulbs consisted of abundant basement membranes whereas the Schwann cells
were few and sometimes lacking. In the 6 biopsies in which teasing was performed signs of present and previous demyelination were noticed. Furthermore, numerous internodal segments were much too thin with reference to their length, which indicates that the neuropathy in these cases primarily causes an atrophy of nerve fibres whereas the paranodal and segmental demyelination are secondary phenomena.

The cause of a nerve fibre atrophy could be either a neuronal or a Schwann cell disorder. Ultrastructural axonal changes were seen in a majority of the cases. Moreover, in some cases there was a marked difference between separate fascicles as to the presence of myelinated nerve fibres, i.e. the loss of myelinated fibres was not as diffuse as would be expected in a primary Schwann cell disease.

The current findings consequently suggest that HMNS I might, in some cases, be an atrophic process primarily affecting the axon.

Immunocytochemical demonstration of human immunoglobulins in the peripheral nerves after systemic passive transfer to mice and monkeys

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A humorally-mediated immunopathogenesis has been proposed in dysproteinemic neuropathy and chronic inflammatory polyneuropathy. In order to investigate whether human IgG can penetrate the intact blood nerve barrier, crude or purified immunoglobulin (Ig) fractions were systematically injected into adult mice and marmoset monkeys. The human Ig or IgG were isolated from patients with dysproteinemic (myeloma) neuropathy, and from healthy volunteers.

The peroxidase-antiperoxidase techniqae was employed for light microscopic demonstration of Ig. In the peri- and endoneurium of all experimental animals, human Ig was observed regardless of its potential pathological significance. Only the corresponding light chain in myeloma-Ig treated animals and both light chains in animals treated with other human Ig yielded positive reaction products in the endoneurium proper. The animals' own IgG was demonstrated only in traces, whereas its albumin gave strong staining in both treated and untreated animals.

The results provide evidence that the xenogenic immunoglobulins may penetrate the intact blood nerve barrier of experimental animals. The interaction between Ig and the blood nerve barrier will be discussed.
Immunocytochemical demonstration of immunoglobulins in sural nerve biopsies

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The results reveal that the blood nerve barrier is normally permeable to albumin, IgG, and both kappa and lambda light chains. In pathological nerve disorders, only slight differences in pattern and intensity of specific immunocytochemical staining was observed. In none of the dysproteinemic neuropathies could increased staining for corresponding heavy and light chains be shown in the endoneurium, whereas moderate endoneural staining for IgM and traces of IgA were seen in 2 of 3 vascular neuropathies. The results and possible factors which may influence the permeability of the blood nerve barrier will be discussed.

Experimental paramyxovirus infections in the mouse brain

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Sendai virus was inoculated i.c. into groups of Swiss albino mice 2, 12 and 21 days of age. In the older mice, there was a rapid rise in antibody titres in the sera and the virus disappeared from the brain within 4 days. In the newborn, 2-day-old mice, the antibody response was delayed and infectious virus was still recovered from the brain 6 days after injection. FITC-labelled polyclonal antibodies to the virus revealed both infected ependymal cells and neurons and that scattered neurons containing viral antigens still occurred 24 days p.i. FITC-labelled monoclonal antibodies to the NP, P, M, F and HN antigens of the virus showed that all these antigens were present in ependymal cells, while the surface antigens, F and HN, were lacking the persistently infected neurons. With the PAP-technique, infected neurons could be seen 8 months after infection. Mumps virus infection in newborn mice produced no infectious virus, but many neurons developed nucleocapsid antigens 9 and 12 days p.i.; this infection also persisted.
Coronavirus-induced demyelinating encephalomyelitis in rats: neuropathological and immunological studies

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Infection of rats with the murine coronavirus JHM can lead to different types of demyelinating encephalomyelitis (DE). The outcome of infection depends on properties of the virus as well as on the age and genetic background of the rat. The incubation time varies between several days to months, and symptoms (paresis, incoordination and paralysis) can show exacerbations, remissions and relapse. The neuropathological changes consists of inflammations, demyelination, and remyelination depending on the stage and type of disease.

Infection of adult Lewis rats with JHM wild-type virus is followed within 8–25 days by DE. Histological changes are perivascular lymphoid infiltrations and demyelinating plaques with infiltration of macrophages. Lymphocytes from infected rats are sensitized against myelin basic protein (MBP) and against virus antigen. Adoptive transfer of lymphocytes after restimulation by culture with MBP is followed by EAE-like lesions in recipient rats. On the other hand, infection of adult BN-rats is not followed by neurological symptoms. The reactivity of lymphocytes from BN-rats against MBP and virus antigen is low, but demyelinating lesions with granulomatous inflammations persists for months. These results suggest that this virus infection can trigger cell-mediated immune reactions against neuroantigens, which could contribute to the development of DE with recognizable symptoms.

Inoculation of young Lewis rats with selected mutants of JHM virus leads to a subacute DE which frequently shows a chronic course characterized by more pronounced demyelination, but fewer perivascular cuffs than found with wild type virus. Rats which develop a relapse of DE. reveal old and fresh demyelinating lesions. Further studies on the pathogenesis of these different courses of DE may contribute to our understanding of virus-induced CNS diseases of man.

Validity of clinical diagnosis in senile dementia: prospective clinicopathological study

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The 2 major forms of dementia in old age are senile dementia of Alzheimer type (SDAT) and multi-infarct dementia (MID). Attempts have been made to correlate clinical evidence with neuropathological findings in these conditions, but only with moderate success. Therefore, we set out to analyse prospectively the accuracy of the clinical diagnosis of dementia. Our earlier
epidemiologic study comprised 421 patients, whose clinical diagnosis was based on the course of the disease, neurological examination with a short psychological test and determination of Hachinski’s ischemic score.

The 58 patients of this study were from the epidemiological material and were a consecutive series of those coming to autopsy. The right half of the brain was fixed and microscopic samples were taken from 15 topographic areas. Bielschowsky preparations were used for quantitative assessment of neurofibrillary tangles and neuritic plaques. SDAT was diagnosed if tangles were present in the neocortex. The diagnosis of MID was made if only ischemic lesions were present; dementia was regarded as combined if Alzheimer-type degeneration was accompanied by any ischemic lesions. The findings are shown in the table. In 4 patients, no specific pathology was detected.

Table
Correlation of clinical and neuropathological diagnoses of senile dementia in 58 patients

| Clinical diagnosis | Neuropathological diagnosis | Sensitivity (of clinical diagnosis, %) | Specificity |
|-------------------|----------------------------|--------------------------------------|-------------|
|                   | SDAT | MID | combined | other |                             |             |
| SDAT              | 20   | 1   | 3        | 4     | 69.0                         | 72.4         |
| MID               | 7    | 7   | 2        | 3     | 70.0                         | 75.0         |
| Combined          | 2    | 2   | 1        | –     | 16.6                         | 90.9         |
| Other             | –    | –   | –        | 6     | 46.2                         | 100.0        |

In conclusion, the ultimate diagnosis of SDAT, MID or combined dementia is a neuropathological one, the bedside clinical diagnosis being only moderately accurate.

The clinicopathological heterogeneity of GM2-gangliosidosis: a propos of an adult with spastic paraplegia, pleomorphic inclusions in enteric neurons and diminished hexosaminidase activity

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The patient is a 22-year-old male living with his parents. These and 4 sibs are reported to be healthy. As a boy he attended a special school. Later on he was an aprentice in a bakery. Right now he is unable to do any work. His health problems are always supposed to have started after an accident of some sort. Thus, stuttering developed at age 4 after a fall; his gait became very bad after a blow on his back at age 16. At present he has dysarthria, spastic paraplegia, brisk tendon reflexes, extensor plantar responses, horizontal nystagmus and kyphoscoliosis. The eyeground is normal. Liver and spleen are not enlarged. Films of stained peripheral blood are unrevealing. The total beta-hexosaminidase activity (hex) of white blood cell homogenates measured with fluorogenic substrates is about 40% of controls. Isoelectric focusing shows a normal distribution of hex with normal isoelectric points of both hex A and B. Heat-inactivation
studies, however, reveal that the patient hex A is more heat-stable than control hex A. In a rectal biopsy specimen, occasional nerve cell bodies and processes of the submucous plexus are stuffed with pleomorphic inclusions. These present a variable mixture of electron lucent and electron dense components, the latter being partly lamellar and partly amorphous. Garden variety membranous cytoplasmic bodies are not seen. Further studies are in progress. Since the spectrum of hexoaminidase deficiency diseases lately became very wide, we think that we are dealing with a new variant of adult GM2-gangliosidosis, the molecular basis of which remains to be elucidated.

Progressive glio-neuronal poliodystrophy in infancy with abnormal mitochondria. Brain biopsy studies in 4 cases

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The pathogenesis of infantile progressive poliodystrophies of the brain (Alpers disease) is obscure as yet. We have investigated brain biopsies in 4 sporadic cases with rather uniform severe clinical course: statomotor and psychic retardation ongoing between 3 weeks and 6 months after birth, muscular hypotonia, later spastic paresis, atactic and athetotic movements, nystagmus, myoclonic jerks and focal seizures, amarousis and optic atrophy. Microcephaly was seen in 3 cases. EEG revealed abnormal rhythmic waves, the CT moderate inner and outer atrophy of the brain. 2 patients died at the age of 9 and 12 months, 2 patients are alive, now 2 and 3 years of age. The brain tissue showed spongi-form changes of grey and white matter and increased numbers of enlarged and polymorphous mitochondria with dense matrix in neurons and astrocytes. Cases with similar clinical picture and histopathology have been reported by Jellinger and Seitelberger under the term “spongy glio-neuronal dystrophy in infancy and childhood”. A genetically fixed deficiency of energy production by neuroectodermal mitochondria is proposed to be one of the pathogenetic mechanisms efficient in this variety of the disease. It is different from Canavan's disease in the type of mitochondrial abnormality and the lack of myelin splitting and vacuolation.
Neuronal changes in the Norrbottian type of Gaucher’s disease

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The brains of 5 cases with the Norrbottian type of juvenile Gaucher’s disease were examined. We have previously reported marked morphological and chemical differences in the degree of glucocerebroside accumulation in the brain between splenectomized and non-splenectomized cases. The 5 cases also exhibited, to a variable degree, signs of nerve cell degeneration and death with neuronophagias. Neurons in most regions also contained granules which were stained for cerebrosides. Ultrastructural examination in 1 case revealed the presence of membranous cytoplasmic bodies (MCB) in Purkinje cells. On the basis of ceramide composition analyses, it has been suggested that the glucocerebroside accumulating in the brain in infantile and juvenile Gaucher’s disease is partly derived from gangliosides.

In summary: (i) widespread neuronal changes of a variable degree are present in the brain in the Norrbottian type of juvenile Gaucher’s disease; (ii) histochemical and ultrastructural findings suggest neuronal storage of glucocerebroside.

Neuronal intranuclear inclusion disease (NIID)

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A pair of identical twins exhibited an unusual combination of neurological symptoms and signs, starting at the age of 11 years with episodes of oculogyral spasms. The subsequent course was characterized by dysarthria, nystagmus, ataxia, fasciculation in the tongue and muscles of the extremities, dysphagia, tremor, rigidity, and grand mal epilepsy. Extensive biochemical, virological and biotic studies were unrewarding. The girls died of pneumonia at the age of 21 years.

At autopsy, almost all types of neurons in the central and peripheral nervous systems as well as in the retina showed acidophilic inclusion bodies in their nuclei. At the ultrastructural level, these inclusions consisted of non-membrane bound aggregates of randomly oriented filaments with a diameter of 9 nm and an electron-lucent core. The inclusions were autofluorescent in ultraviolet light, showed a strong histochemical reaction for aromatic amino acids, and were extremely resistant to detergents. Immunohistochemical studies excluded the presence of actin.

We propose the designation neuronal intranuclear inclusion disease (NIID) for this condition. NIID apparently represents a unique type of inherited disease with intranuclear accumulation of the products of deranged metabolism.
Do lymphocytic structural abnormalities exist in the carrier state of juvenile ceroid-lipofuscinosis?

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Peripheral blood lymphocytes with cytoplasmic vacuoles are a characteristic finding in children suffering from the juvenile type of generalized ceroid-lipofuscinosis. By electron microscopy, the vacuolated lymphocytes reveal osmiophilic inclusions mostly with a fingerprint-like pattern rendering these abnormalities reliable morphologic markers for diagnosis of the disease. However, controversy exists as to whether vacuolated lymphocytes also occur in clinically healthy relatives of affected children providing a useful indicator of the carrier state. It is also disputed whether lymphocytes of carriers reveal ultrastructural inclusions or an increase of so-called parallel tubular arrays (PTA), a genuine cell organelle of a subpopulation of peripheral lymphocytes. We have re-evaluated this question electron microscopically in 15 parents of affected children and in 5 normal siblings of one affected child. Peripheral blood lymphocytes of normal persons served as controls. Neither abnormal vacuolated lymphocytes nor an increase of PTA could be found in the carrier state of generalized ceroid-lipofuscinosis. It is concluded that screening of lymphocytes is not suitable for detecting heterozygotes of the juvenile type of ceroid-lipofuscinosis.

Brain weight and volume of lateral ventricles in male alcoholics

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Multivariate regression analysis to explain the variation in brain weight of 45-54 and 70-79 year-old males, showed a significant decrease in the weight of the cerebral hemispheres and of the cerebellum and brainstem in alcoholics. Cases with alcoholic cerebellar atrophy showed a more severe weight reduction of the cerebral hemispheres than alcoholics without cerebellar atrophy \((P < 0.025)\), thus indicating a common cause of the cerebral and cerebellar damage. Young alcoholic males had larger ventricles than non-alcoholics. Alcoholics with cerebellar atrophy had a lower body mass index than those without cerebellar atrophy \((P < 0.05)\), thus suggesting a nutritional factor as a possible cause of brain damage in alcoholics.
Extent and severity of the brain lesions in Wernicke’s encephalopathy

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A previous study showed that (i) coma was the dominating symptom in most cases of active (acute) Wernicke’s encephalopathy, and (ii) that most of the cases with inactive (chronic) disease showed a global dementia rather than Korsakoff’s psychosis. These findings prompted a reexamination of the brain lesions in this disease.

A study of 30 cases of Wernicke’s encephalopathy, 15 of which were active and 15 inactive, showed a decreasing vulnerability of the brain tissue from the mammillary bodies downwards through the medial thalamus and into the brainstem.

Only 2 of the cases with active disease had lesions restricted to the mammillary bodies. The others had simultaneous involvement of the thalamus. Involvement of the brainstem was seen in some but not all cases. This may explain the frequent lack of brainstem symptoms in such cases.

Most cases of inactive disease had lesions of varying severity restricted to the mammillary bodies. Simultaneous involvement of the thalamus was seen in only 3 cases. These findings explain the variations in the memory deficit but not the global dementia. Additional lesions, e.g. alcoholic brain atrophy, are probably present in such cases.

Cellular damage after chronic phenytoin administration.
An electron microscopic investigation of mouse cerebellum*

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Male C57B16J mice were housed individually in plastic cages and were fed the vitamin-supplemented liquid diet “Stardit”. 10 experimental animals received phenytoin in their diet. During the first 2 weeks of administration, the drug content of the diet was steadily raised until the final concentration was reached. At that time each experimental animal received the daily dose of 150 mg phenytoin/kg body weight. The serum-phenytoin levels of experimental animals were 15–25 µg/ml.

After 4 weeks, experimental animals developed ataxia and after 6 weeks 10 experimental animals and controls were perfused with glutaraldehyde and prepared for electron microscopy. Probes of the cerebral cortex (area 3) cerebellum, thalamus, hypothalamus and liver were embedded in araldite. Semi-thin sections and electron microscopy of the cerebellar vermis showed dystrophic changes in Purkinje cell axons, alterations of Purkinje cell dendrites and pycnoses of granular cells. Furthermore, experimental ani-
mals displayed a drug-induced hepatopathy with proliferation of the endoplasmic reticulum. Similar membranous proliferations were observed in some Purkinje cell dendrites. It is suggested that the high binding capacity of cerebellar tissue to phenytoin and a drug-induced disturbed protein biosynthesis might be responsible for the cellular damage.

* This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG).

Cytological localization and toxicity of adriamycin in the nervous system

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The distribution of the powerful cytostatic drug adriamycin (doxorubicin) in the nervous system of the mouse, was investigated following various routes of administration. The drug was localized by a direct fluorescence microscopic technique after intravenous, intraventricular, endoneurial and intramuscular injections.

The blood-brain and the blood-nerve barriers prevented detectable amounts of the drug entering most of the brain parenchyma and the endoneurium of the sciatic nerve. Adriamycin after intravenous injection gained access to regions with permeable blood vessels such as the choroid plexus, the lamina cribrosa of the optic nerve, the circumventricular organs and the peripheral somatic and autonomic ganglia. In all these regions, the drug was bound to the nuclei of neurons and glial cells, which became fluorescent.

Light and electron microscopic investigations were conducted to ascertain whether this deposition of adriamycin produced cytotoxic damage in the circumventricular organs. Nuclear and cytoplasmic changes were observed in the neurohypophysis, median eminence and postremal area, the regions so far examined. Because adriamycin is widely used to treat malignant disease, further studies should be undertaken to see whether such changes occur in man.

Adriamycin was found to enter motor and sensory nerve terminals and undergo retrograde axonal transport to their corresponding nerve cells in the nervous system. The drug injected into the tongue was transported to the neurons of the hypoglossal nucleus, where it labelled the nuclear chromatin and induced progressive degenerative changes.

The drug may be used as a fluorescent intracellular tracer which produces selective nerve-cell degeneration. A toxic model for the experimental study of motor neuron disease is thereby introduced.
Distribution of cadmium in the nervous system of the rat

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Acute cadmium intoxication causes haemorrhagic and necrotic lesions localized selectively in the sensory ganglia of adult rodents. In newborn rats and mice, the injection of cadmium produces hemorrhagic encephalopathy due, it is thought, to a primary action of cadmium on the endothelial cells.

In the present study, adult rats were given an intravenous injection of radioactively-labelled cadmium ($^{109}$Cd) and the distribution of the isotope in the nervous system was subsequently studied by autoradiography. Cadmium accumulated in regions without the blood-brain-barrier such as the choroid plexus, pineal gland and area postrema but did not appear in the brain parenchyma. Uptake of cadmium was observed in the trigeminal ganglia close to the nerve cells and in the olfactory bulbs. In addition, cadmium had accumulated in the iris, ciliary body and choroid of the eye but not in the optic nerves.

The deposition of cadmium in the olfactory bulbs may explain the anosmia reported in workers exposed to cadmium dust. The deposition of cadmium in restricted regions of the brain may have relevance for some of the neurotoxicological effects which have been reported in animal experiments.

Low dose lead encephalopathy in normal and protein deprived suckling rats

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An attempt was made to further elucidate the association between lead encephalopathy and growth retardation. The growth-retarded offspring of protein-deprived rat mothers (PD rats) were exposed to 10 or 5 mg lead nitrate/kg body weight daily for 15 days post partum. These doses of lead do not provoke growth retardation in normal suckling rats but the higher dose (10 mg/kg b.wt. daily) results in light microscopical signs of encephalopathy. The PD rats given 10 mg/kg b.wt. daily developed devastating brain lesions. Growth retardation as compared with unexposed PD rats was apparent from 10 days and the mortality was close to 100% between day 15 and 20. PD rats given 5 mg/kg b.wt. daily also presented light microscopical signs of encephalopathy though less devastating. This group showed no additional retardation of body growth and the mortality was only some 20% between days 15-20. The results imply an increased vulnerability to lead in growth-retarded offspring of protein-deprived mother rats.
Light-microscopical and ultrastructural aspects of bilirubin encephalopathy in the Gunn rat

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Gunn rats are a Wistar substrain, in which an enzymatic defect of glucoronyltransferase activity leading to neonatal jaundice is inherited as a recessive trait. The homozygous animals of this strain are subject to bilirubin encephalopathy with resulting neurological symptoms such as ataxia and dysequilibrium.

The influence of bilirubin on cerebellar growth was studied at various ages and under varying experimental conditions. High bilirubin levels cause persistent reduction in cerebellar size and loss of Purkinje and granular cells. Phototherapy can prevent these neurotoxic effects. Sulfonamide application results in an almost complete cessation of cerebellar growth.

Up to the 8th day after birth, the cerebellum of jaundiced animals does not differ macroscopically from those of controls. Microscopically the Purkinje cells show varying degrees of swelling and vacuolization of the cytoplasm, and granular inclusions. In the first day of life, the inclusions consist of loosely arranged whorls of membranes. At 14 days of age, the irregularly shaped membranous bodies are diminished in number. Membrane-bound aggregations of glycogen particles predominate, most of them located inside mitochondria.

Glycogen accumulations in mitochondria can be seen from 8 days onwards, up to 12 months, suggesting that uncoupling of oxidative phosphorylation is the primary biochemical event underlying bilirubin cytotoxicity.

No abnormalities were found in heterozygous animals or in animals treated by phototherapy.

Angiogenic tumors of the nervous system – an immunohistochemical evaluation

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The distribution of endothelial cell and basement membrane (BM) markers were studied in 17 cases of hemangioblastoma, hemangioendothelioma and hemangiopericytoma of the CNS, using immunofluorescence and immunoperoxidase techniques. Ulex Europaeus lectin (UEA I) and factor-VIII-related antigen (FVIII RAG) were used as endothelial cell markers, and laminin as BM marker.

It was found in all tumors that UEA I and FVIII RAG only bind to endothelial cells. As the stromal cells of hemangioblastoma did not
express these endothelial cell markers, our results do not favor the view that the stromal cells are derived from endothelial cells. In hemangioendotheliomas, UEA I was a more sensitive endothelial cell marker than FVIII RAG, as UEA I bound to all endothelial cells while anti-FVIII RAG only bound to endothelial cells forming vessel lumina.

Laminin was only found in the BM of vessels, not in the interstices of the neoplastic cells. Therefore, the reticulin network of hemangio-pericytomas does not correspond to BM. The staining pattern of each tumor was characteristic, which suggests that laminin could be of use in the histological diagnosis of the angiogenic tumors of the nervous system.

Glial fibrillary acidic protein (GFAP) in mixed gliomas (MG)

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39 mixed gliomas were investigated histologically and immunohistochemically. The method of Sternberger was used for the immunoperoxidase demonstration of GFAP in paraffin-embedded 6 μm thick sections.

Results
Morphologically, the MG can be divided into (3) groups.
(i) The astrocytic cells (AC) were seen intermingled with oligodendroglia cells (OC) (28 of 39; 72%).
(ii) The tumor is made up of clearly delimited astrocytic and oligodendromatous areas (10 of 39; 25%).
(iii) The tumor cells are intermediate from between AC and OC (1 of 39; 3%)
The GFAP-positive reaction sites and frequency were as following.

Intermediate cells: transitional forms which are GFAP-positive were observed in 14.56% of the MG.
Astrocytic cells: cytoplasm 82.04%; perinuclear rim 7.69%; small intracytoplasmic droplets 7.69%; end-feet 7.69%. Negative AC were seen in 56.40 of the MG.
Oligodendroglia cells: cytoplasm 35.89%.

Conclusions
(a) Histochemically, the MG can be divided into the 3 major abovementioned groups.
(b) Transitional elements from oligodendroglial cells into astrocytes, or the other way round, seem to occur in Mg.
(c) Frequently observed were AC being GFAP-negative and OC showing GFAP activity.
(d) In Mg there are several GFAP location sites in different glial cells.
Patterns of intermediate filament expression in cultured human glioma cell lines and primary cultures

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The intermediate filaments in detergent-resistant cytoskeletons of cultured human glioma cell lines and primary cultures were studied by scanning and transmission electron microscopy, immunofluorescence microscopy, SDS-polyacrylamide gel electrophoresis and immunoblotting technique. In the glioma cell lines, the major cytoskeletal polypeptides with molecular weights of 51 and 58 kd were identified as glial fibrillary acidic protein (GFAP) and vimentin. Neither protein could be detected in the detergent extracts of the cells. In the primary cultures, the majority of the cells also showed co-expression of GFAP and vimentin. However, in a portion of the cells in these primary cultures, GFAP could be observed as the only intermediate filament protein in double immunofluorescence stainings. These cells disappeared during subcultivations.

Thus GFAP and vimentin are both included in the detergent-resistant cytoskeleton of cultured human glioma cell lines, with no clearly detectable soluble pool of either protein. In the primary cultures, GFAP can occur as the only intermediate filament protein in a portion of the cells. These results prove that GFAP shares most of the characteristics of intermediate filament proteins found in other cells.

Phleomorphic xanthoastrocytoma – report of 5 cases

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5 cases of pleomorphic xanthoastrocytoma are described. The favourable prognosis of these tumours is confirmed. Thus, 2 patients had post-operative survivals of 9 and 12 years, respectively, and 3 others are still alive and well, one of them 6 years after operation.

All the cases that have been described until now (17, when the present ones are included) have occurred in patients below 30 years of age. Most of the tumours have been located superficially in the brain with extensive adhesions to the meninges, and usually they have been well demarcated from the brain.

Histologically, the tumours show a marked cellular pleomorphism, including bizarre giant cells, but there are few mitoses and only a slight tendency to necrosis. The tumour tissue contains a dense network of reticulin fibres and many cells contain lipid vacuoles. Glial fibrillary acidic protein (GFAP) can be demonstrated in the cytoplasm of the tumour cells.
Immunocytochemical identification of factor VIII and Ulex Europaeus Type I lectin binding in endothelial cells in human vascular meningiomas

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Factor VIII, a glycoprotein present in normal human plasma, and Ulex Europaeus Type I lectin (UEA I) are considered as markers for endothelial cells. These 2 markers were visualized by an unlabeled peroxidase-anti peroxidase technique (PAP) and an avidin-biotin-peroxidase-complex method (ABC) for factor VIII and UEA I, respectively, in paraffin sections of selected cases of human meningiomas and normal brain. Specific staining of venules, small arteries and capillaries was equally well achieved by both markers, whereas small capillaries could be seen more often with UEA I. With higher concentrations (0.002 to 0.01 mg/ml) of UEA I, slight to moderate staining of erythrocytes prevailed. This is in contrast to other studies, using the less sensitive immunofluorescence technique. In small areas of a few angiomatous and fibroblastic meningiomas small cells within the tumour tissue appeared positive.

The nature and origin of these cells will be discussed.

Transcortical motor aphasia following left frontal neoplasm
A case study

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Frontal lobe damage often leads to serious cognitive and emotional disturbances. These disturbances depend on the site, size, course and etiology of the lesion. We describe a detailed clinical, neuropsychological, neuroradiological and neuropathological examination of a patient with a rarely-reported aphasic syndrome. The initial symptoms were moderate headache, absent-mindedness and difficulty in spontaneous speech. Neurological status was unremarkable, but in neuropsychological examination the mental deficits were diagnosed as transcortical motor aphasia (TMA). It is a specific syndrome characterized by well-preserved auditory comprehension and repetition, whereas initiation and maintenance of speech and other cognitive functions are disturbed. This syndrome is seen in left frontal lesions just anterior to, superior to or deep within the Broca's area. In the present case, CT scan showed a tumour located posteriorly in the left frontal lobe. The findings on the CT and on carotid angiography were characteristic of a malignant glioma. The CT also revealed a hematoma within the neoplasm. Neuropathological findings were typical of glioblastoma. The present case of TMA exemplifies the importance of detailed neuropsychological assessment in the diagnosis of neurologically "silent" brain tumours.
Hypoglycemic brain injury: localization of permanent neuronal damage in a rat model allowing long-term recovery

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Study of purely hypoglycemic brain damage requires an adequately monitored animal model to exclude hypoxia, hypotension, and epilepsy as factors contributing to the damage. Study of permanent cell damage constituting neuronal necrosis requires a model allowing long-term recovery, due to the reversible nature of some early pathologic lesions, and the maturation of other pathological lesions into neuronal necrosis. A model fulfilling both these criteria has been developed in the rat. After one week survival, localization of neuronal necrosis was remarkably selective, affecting several gray matter structures in the following order: caudate nucleus, dentate gyrus, cerebral cortex, and the CA 1 sector of the hippocampal pyramidal layer. Within the dentate gyrus, a sub-localization was seen, with the crest of the gyrus affected before the internal and external blades, and the anterior portion affected before the more posterior portions. A sub-localization was also seen in the cerebral cortex, with damage almost exclusively localized to layers 2 and 3.

Vascular malformations in the nervous system in psychiatric patients

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Examination of the nervous system of psychiatric patients may give unexpected findings. In one year we have examined the brains of 114 psychiatric patients; vascular malformations were found in 6 of these. 5 of the patients were more than 70 years old. One of these had a capillary angioma, 3 had localized capillary telangiectases, while the 5th had an arterio-venous malformation. The 6th patient was a 31-year-old man with telangiectases of the central and peripheral nervous system.
Ischemic damage of hippocampal CA-1 neurons. Possible neurotoxicity of glutamate released during ischemia

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A short period of transient cerebral ischemia damages the hippocampal CA-1 region (selective neurone loss) in particular. Counting of pyramidal cells and interneurons in the rat hippocampal CA-1 region have shown that pyramidal cells are selectively lost, whereas interneurons in stratum oriens are resistant to transient cerebral ischemia for a period of up to 20 min. Ultrastructural studies of stratum radiatum show loss of and pronounced damage to the dendrites of CA-1 cells, whereas the presynaptic structures (predominantly belonging to Schaffer collaterals) are undamaged. The Na+-dependent high affinity uptake of glutamate (in terminals) in CA-1 is also unchanged after ischemia. The selective neuron loss might be caused by an excessive release of neuroexcitatory transmitter, e.g. glutamate, during ischemia. Thus, amino acid analyses of hippocampal dialysates (in vivo), obtained using a 300 μm dialysate fiber revealed pronounced elevations of extracellular glutamate and aspartate immediately after the onset of ischemia, whereas only minor effects on other amino acids were seen. This may explain why EM studies with horseradish peroxidase (HRP) as the extracellular tracer show a high postischemic permeability of the dendritic spines selectively at the postsynaptic level. Since dendrites are known to have increased Ca2+ conductance during ischemia, a possible damaging effect of glutamate and/or aspartate under ischemic conditions could be mediated by impaired Ca2+ sequestration in the dendrites.

Ultrastructural changes in kaolin-induced hydrocephalus in the rat

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Alterations of the microvasculature in the subependymal white matter were studied by electron microscopy in rats with experimental hydrocephalus. Normal adult rats were used as controls. After injection of kaolin-suspension into the cisterna magna, the animals were killed at intervals of 1, 2, 4, and 8 weeks. All of these showed hydrocephalus in varying degrees. 1 week after kaolin-induction of hydrocephalus, there was a mild increase in the extracellular space in the neuropil. In 10 of 33 vessels examined (30%), there were dehiscences in the
interendothelial cleft between the tight junctions. At 2 and 4 weeks of hydrocephalus, the interendothelial spaces were more numerous (24/42: 60% at 2 weeks, 18/35: 55% at 4 weeks), and some of these were enlarged, forming interendothelial blisters. After 8 weeks hydrocephalus, dehiscences such as those seen after 1 week were still prominent (13/30: 43%), but the number of endothelial blisters had decreased. In rats that had been injected intravenously with horseradish peroxidase (HRP), the interendothelial blisters were completely devoid of the marker substance. These findings indicate that in obstructive hydrocephalus, the tight junctions may constitute a part of a paracellular shunt pathway for the resorption of interstitial edema fluid in the presence of increased intraventricular pressure.

Local intra-arterial fibrinolytic therapy in the vertebro-basilar system: preliminary post mortem findings

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Thrombotic or thrombembolic occlusions in the vertebro-basilar system have a poor prognosis: a fatal outcome (86%) or severe neurological deficits have been reported in 282 published cases. In acute thrombotic occlusions of the vertebro-basilar arteries, thrombolytic therapy may be effective while transluminal angioplastic procedures or reconstructive activities have not been applied. Systemic intravenous streptokinase administration is presently disapproved because of the risk of hemorrhages into the infarcted cerebral areas. However, local intra-arterial fibrinolytic therapy (LIFT) using streptokinase, for the first time applied by Zeumer via catheter into the vertebral artery, had good success in 6 of the 9 cases treated thus far.

The post mortem findings in the first 3 autopsied patients following LIFT will be reported. An intracerebral hemorrhage as a result of the therapy was thus far not observed; lesions of the vessel walls, however, were focally encountered. The limiting factors for recovery appear to be the age and size of the actual necrosis and edema, the degree of arteriosclerosis, and the arrangement of the collaterals.
Neuronal uptake of extravasated macromolecules of horseradish peroxidase and their transport by axons to other regions from a focal vasogenic cerebral edema

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A study was made of acute nerve cell reactions in and remote to a cryogenic lesion of the frontal cortex of adult mice injected intravenously with horseradish peroxidase immediately after making the lesion. The brain and segments of the cervical, thoracic and lumbar spinal cord were examined 3, 12, 24 and 72 h later by light microscopy after being subjected to diaminobenzidine and tetramethylbenzidine histochemical techniques.

HRP was found in axons on both sides of the corpus callosum and in the ipsilateral internal capsule, cerebral peduncle, pyramidal decussation and the contralateral pyramidal tract of the cervical spinal cord.

Neurons of the contralateral frontal cortex and ipsilateral ventral and medial thalamic nuclei and pars compacta of the substantia nigra contained HRP: it was found also in axonal terminal fields in the ipsilateral subthalamic nucleus and the reticular part of the substantia nigra and the pontine nuclei.

This study indicates that neurons remote from focal vasogenic brain edema may accrue and be influenced by material taken up by nerve cells and axonal terminals in the primary lesion. Such substances may undergo axonal transport, somatofugally as well as somatopetally directed.

The presence of small intensely fluorescent (SIF) cells in the leptomeninges

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Small intensely fluorescent (SIF) cells have been reported in the sympathetic ganglia, some nerves, carotid body and visceral organs. They are characterized by high monoamine content. This study includes autopsy material from 40 individuals (16 males and 24 females, 21 to 75 years old); none of them had disease of the central nervous system. In addition, 10 human fetuses were also used. Electively, fragments of the leptomeninges corresponding to the frontal lobe, praecentralis gyrus, and fissura calcarina were studied. Histofluorescence for indoleamines
(IA) and catecholamines (CA) was induced according to the 3 following procedures: formaldehyde vapour, sucrose-phosphate buffered glyoxylic acid solution and Faglu fixation. In the leptomeninges of adults, SIF cells were easily found. They are spherical or oval when isolated and polyhedral when clustered. The size of the cluster is highly variable, from groups of 4 or 5 to 50 or even more cells. They were mostly situated close to the blood vessels. They exhibit an intense yellow fluorescence which is homogenous in the cytoplasm, the nucleus being hardly distinguishable. Sometimes isolated cells exhibiting an orange-yellow fluorescence may be seen inside the clusters. In addition, bipolar cells of greater size exhibiting a granular green-yellow fluorescence can also be detected along the blood vessels. In fetuses, unexpectedly, no fluorescent cells could be found.

Monoclonal antibodies to intermediate filaments in neuropathology

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Immunocytochemical techniques in combination with monoclonal antibody technology are already revolutionizing some areas of pathology, and a new concept of antigenic histology is emerging. Monoclonal antibodies (Mab) have been produced to intermediate filament proteins, glial fibrillary acidic protein (GFAP), the neurofibrillary protein triplet (NFP 200 kd, 150 kd and 68 kd) and tubulin, using the Balb-c mouse system. The have been characterized immunocytochemically, biochemically and immunocytochemically. The GFAP-Mab is an IgG1 molecule while all others are IgM molecules. These Mabs have been applied extensively to cultured cells and normal human tissues. The GFAP-Mab has been used in the histological and cytological diagnosis of human brain lesions and has been found to be of assistance, particularly in tumor diagnosis. The NFP-Mabs are currently being evaluated for their value in distinguishing the abnormalities of neurofilament arrangement found in different syndromes. The antibodies have also been utilized in basic studies of intermediate filament function. In contrast to antisera production, Mab technology provides specific antibodies in unlimited quantities which may be utilized under standardized conditions. Direct conjugation of marker enzymes or fluorochromes to these Mabs should make their use as simple as todays routine stains.
Structural requirements for receptor-mediated endocytosis of glycoproteins in nerve cell culture

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Virus entry into tissue cells is, under physiological conditions, thought to be mediated by high-affinity binding to receptors on the cell surface, with subsequent internalization of the receptor-virus complex. It is, however, still not clear whether viral glycoproteins participate in virus entry and if so, whether the structure of the carbohydrate chains specifies virus adsorption and tissue preference of infection. In order to question this rôle of viral glycoproteins, orosomucoid (α1-acid glycoprotein), a plasma glycoprotein containing several complex-type carbohydrate chains N-glycosidically bound to the polypeptide, was used as a ligand since this type of carbohydrate structure is commonly occurring on viral capsid glycoproteins. Exoglycosidases (β-galactosidase and β-N-acetylhexosaminidase) and endoglycosidase D were used to sequentially and specifically remove different sugars composing the N-glycan of orosomucoid. These treatments produced derivatives of orosomucoid with different carbohydrate composition and exposing different terminal sugars. The derivatives were conjugated with horse-radish-peroxidase (HRP) and the uptake of the different HRP-conjugated glycoprotein derivatives was studied on dorsal root ganglia cells in primary culture. In this system, the nerve cell bodies inside a cloning cylinder are isolated from the neurites by a diffusion barrier. Binding of the HRP-glycoprotein conjugates was carried out at 4°C for 4 h and uptake was at 37°C for 18 h; it was demonstrated that the neurites preferentially recognized galactose-terminated derivatives of orosomucoid and translocated this HRP-conjugate to the cell body.

Neuron-associated peptides in human colonic biopsies

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Using a modified unlabeled antibody method, the occurrence of neuron-associated peptides SRIF, METE, LEUE, VIP, tubulin and MBP in the human colonic wall was investigated (in addition to histological normal tissue, 9 suction biopsies of Hirschsprung’s disease (HD) and 1 case of a vesicointestinal fissure (VIF)). A co-localization of spotted VIP-IR and ACHE-
activity in the pathognomonic nerve fibers of the lamina propria could be demonstrated in HD. The tubulin antiserum stained neuronal structures such as the cytoplasm, the axon cone and axons preferentially within the deep muscle layers of the gut wall and the submucosal plexus. Neither endocrine mucosal cells nor aberrant fibers in the lamina propria in HD and VIF showed tubulin-IR. This property could prove the tubulin antibody to be an appropriate tool in detecting neurons of the deep plexus suitable for defining the borderline of the resection of the aganglionic segment in the surgical treatment of HD. A similar staining pattern was found in MBP-IR, demonstrating myelinated fibers and intestinal glial cells mainly within the deep plexus, and this serum could attribute to further differentiation of neuronal intestinal dysplasia syndromes with respect to Schwann cell hyperplasia. In the case of VIF, numerous normal reacting ganglionic and glial cells could be demonstrated in the submucous and myenteric plexus, but pathologically as in HD there could be found aberrant VIPergic-cholinergic fibers in the mucosal lamina propria.

Comparative morphometric studies of the post-natal development of peripheral nerves

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A morphometric study of the n. ischiadicus in 2 species of mice was started to compare these finding with the facts known about human developing nerves from nerve biopsies and autopsies. First we compared nerves from a nidifugous animal (spiny mouse, Acomys cahirinus) with those of a nidicolous animal (C3H mouse). The measurements were performed light microscopically on photographs of semi-thin sections at a magnification of 3000 × with a Videoplan (Kontron), and on the ultrastructural level by counting the myelin lamellae on electron-microscopic photographs. In constant areas, the myelinated nerve fibres were counted and the following parameters were determined: areas and perimeter of the whole fibre and of the axon, thickness of the myelin sheath and mean diameter of the whole nerve fibre. We looked for correlations between areas and perimeters and the thickness of the myelin sheath. In most developmental stages, the best correlation existed between the perimeter of nerve fibres and the myelin sheath thickness. The slopes of the regression lines move rhythmically up and down and in adult animals reach a lower level than at earlier stages.