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**Chairman: Professor
Werner Schröter**

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SPECIFIC CELL-MEDIATED IMMUNE RESPONSES AFTER VACCINATION WITH AN ACCELLULAR PERTUSSIS VACCINE OR WITH WHOLE-CELL PERTUSSIS VACCINES IN COMPARISON TO NATURAL INFECTION. M. Knuf, P. Habermehl, H. J. Schmidt, W. Mannhardt, P. Schmidtke, F. Zepp

Background: Pertussis is a two stage disease in which bacterial attachment to respiratory mucosa is followed by pharmacological effects mediated by various toxins. Currently, acellular Pertussis vaccines (DTPa) containing adhesins and detoxified toxins are intensively studied, with the objective of achieving protection while minimizing adverse effects attributed to whole-cell Pertussis vaccines (DTPw). To date there is little understanding of the role of toxins in human disease and still less evidence that antitoxin immunity takes any part in the prophylaxis of whooping cough. Also nothing is known on the nature of cellular immune response emerging in vaccine recipients. The aim of this study was the investigation of the specific cell-mediated immune (CMI) responses induced by DTPa, DTPw and to compare these data to immunity after natural infection.

Methods: The capacity of peripheral blood T-lymphocytes to respond to the pertussis related antigens pertussis-toxin (PT) and the adhesins FHA and 69 kDa-protein was investigated in children before and after vaccination with DTPa or DTPw by measurement of antigen-specific proliferation, lymphocyte phenotype, cytokin production and expression of activation markers (CD25, CD45RO, CD71, HLADR). The results were compared to findings in children 4–6 weeks after recovery from pertussis infections.

Results: Before vaccination only PT possessed unspecific mitogenic properties. Vaccination with the DTPa created a specific T-cellular response to PT, FHA and 69 kDa that was shown to increase continually, depending on the progress of the vaccination schedule. The presence of a definite PT-specific response was proven by testing a non-mitogenic recombinant PT. Phenotype analysis revealed predominant activation of CD4⁺-T-helper-cells and an elevation of CD45RO probably representing memory-induction. Measurement of cytokin-pattern showed primary induction of IL2/IFN γ – producing T-helper-1- with low stimulation of IL4/IL10 producing T-helper-2-cells. CMI responses after DTPa were superior to responses after DTPw and equaled those after natural infection.

Conclusion: Our data indicate that DTPa-vaccination induces a potent immune response to PT, FHA and 69 kDa that is superior to CMI after DTPw and equivalent to the specific cellular immune response after of natural infection. The findings not only have implications for the future development of pertussis vaccines but also elucidate our understanding of the pathogenesis and relevant immune responses in pertussis infections.

Universitäts-Kinderklinik, Pädiatrische Immunologie, Langenbeckstraße 1, D-55101 Mainz

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MOLECULAR CHARACTERISATION OF A NEW EPSTEIN-BARR VIRUS TYPE 1 SUBSTRAIN. V. Schuster*, S. Seidenspinner, H. W. Kreth

EBV, the infectious agent of infectious mononucleosis, is associated with certain malignancies (Burkitt lymphoma, Hodgkin disease, B and T cell lymphoma, nasopharyngeal carcinoma) and B cell lymphoproliferative

disorders. Two distinct EBV types (EBV-1, EBV-2) exists. Little is known about the frequency of mutant EBV strains in vivo and whether they are associated with certain clinical conditions.

Methods: EBV isolates from 9 EBV-associated malignant tumours and from lymphatic tissue of two siblings with chronic lymphoproliferative EBV infection were analysed and genotyped in the EBNA2 gene (direct sequencing) and in the Epstein-Barr early region (EBER; SSCP analysis, direct sequencing), which is 40 kb apart from EBNA2. [EBNA2 plays an essential role in the transformation of B lymphocytes by EBV and seems to be a critical determinant for EBV-induced lymphoma tumour growth. The role of the EBER gene is unclear.]

Results: 4 EBV isolates (angioimmunoblastic lymphadenopathy ($n = 2$), Hodgkin disease ($n = 1$), B cell lymphoma ($n = 1$)) exhibited 6 identical point mutations and an insertion of three nucleotides (gag) within the EBNA2 gene when compared with EBV-1 strain B95-8. EBV isolates from two siblings (boy, girl) who both died from B cell lymphoproliferative disease, revealed similar point mutations and additionally a 51 bp deletion within the EBNA2 gene. All isolates were found to contain EBV-2 sequences within the distant EBER gene. Other EBV isolates from lymphoma (T cell lymphoma ($n = 2$), B cell lymphoma ($n = 3$)) and from benign lymphatic tissue (tonsils ($n = 5$), peripheral blood ($n = 5$)) revealed the "normal" EBV1 sequence pattern as found in EBV-1 strain B95-8.

Conclusions: We describe a new EBV1 substrain which carries characteristic mutations within the EBNA2 gene and EBV-2 sequences in a 40 kb distant viral gene (EBER) suggesting to be a recombinant between type 1 and type 2 virus. It remains to be elucidated if this mutant EBV strain is restricted to malignant tumours. A related EBV1 substrain was found in two children with fatal EBV-induced lymphoproliferative disease. We suggest that the described mutations/deletions within the EBNA2 gene may have altered the biological properties of the virus and may have directly influenced the clinical course.

Children's Hospital, University of Würzburg, D-97080 Würzburg, FRG

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EFFECT OF THE RECOMBINANT HUMAN HYBRID INTERFERON ALPHA B/D (α B/D) ON HSV-1 REPLICATION IN THE MOUSE SYSTEM. U. Wintergerst*, E. Kern, R. Whitley, D. Gangemi, S. Chatterjee

Acyclovir (ACV), the standard treatment for human Herpes-simplex encephalitis, has a remaining mortality of 20% and a defect healing rate of 50%. Interferons are potent antiviral drugs, which may offer therapeutic benefits in combination with ACV. In this study mechanisms of action on the replication of HSV-1 in mouse fibroblast cells (3T3) and in vivo efficacy on experimental HSV-1 encephalitis in mice were investigated.

3T3 cells were treated with different concentrations of interferons (α B/D; mouse interferon, m-IFN) and infected with HSV-1. 24 h after infection plaque assays were performed on BSC-1 cells. Ultracentrifuged supernatants and cell lysates were subjected to dot blot and PAGE-SDS analysis with a human anti HSV-1 antibody and monoclonal antibodies (moAB) against the viral glycoproteins gB and gD. Weanling Swiss Webster mice were intranasally infected and treated (24 hours p.i.) with various doses of α B/D, m-IFN and ACV for 5 days. Mortality was checked until day 21.

α B/D inhibited HSV-1 replication in 3T3 cells. The EC_{50} was 85 IU/ml and only 3 times less than with natural mouse interferon (m-IFN). Dot blot analysis of ultracentrifuged supernatants demonstrated the release of a significant amount of non-infectious particles in α B/D treated, but not in m-IFN treated cells. Immunoblots of cell lysates with anti-HSV-1 showed that α B/D, in contrast to m-IFN, did not suppress the production of the major capsid proteins. Electromicroscopy of HSV-1 infected α B/D treated cells displayed an amount of nucleocapsids in the nucleus similar to the untreated control. However, in immunoblots with moAB against the essential glycoproteins gB and gD a dose-dependent decrease was observed in α B/D treated cells. No toxicity was seen with concentrations up to 10,000 IU/ml. In conclusion, the human interferon α B/D has antiviral effects in mouse cells and acts on the late stage (glycoproteins processing) of HSV morphogenesis.

The good effect of this interferon in vitro corresponded to a reduction of mortality in intranasally HSV-1 infected mice. $2 \times 100,000$ IU/day α B/D reduced significantly ($P < 0.05$) the mortality from 100% to 67%, whereas m-IFN had almost no effect (to 83%). In combination with suboptimal doses of ACV, α B/D reduced the mortality a further 20% compared to the respective ACV control group. Our data might stimulate the discussion on combination therapy of ACV with interferon in HSV encephalitis in children.

* University Children's Hospital, Lindwurmstr. 4, D-80337 München

4 EPITOPES OF α_{S1} -CASEIN RECOGNISED BY HUMAN IGE. H. Müller, P. Spürgin, M. Walter, M. Brandis, J. Forster

Background: α_{S1} -casein, nearly absent in human milk, but the major protein in cow's milk, is one of the major allergens of cow's milk. Little is known about the epitopes on this protein which are targets of human IgE antibodies. The aim of this study was to identify the B-cell epitopes of α_{S1} -casein by an ELISA method based on synthetic peptides as antigens.

Method: A set of decapeptides shifted by one amino acid according to the known amino acid sequence of bovine α_{S1} -casein was synthesized on polyethylene pins (Cambridge Research Biochemicals) using the Fmoc-method. Peptides were screened with sera from fifteen cow's milk allergic children (with positive challenge and skin-prick test) using an ELISA to human IgE, comprising successive incubation with three antibodies, the last one labeled with horseradish peroxidase. In the same way sera from three negative controls and IgE-negative cord blood were analyzed.

Results: All sera of cow's milk allergic children showed a high reactivity with peptides representing amino acids 19–30, 93–98 and 141–150 of the bovine α_{S1} -casein-sequence, indicating common epitopes. The highest reactivity in positive sera was found in the amino acid sequence 141–150. Additionally, individual sera reacted with other different regions indicating individual epitopes. The common epitopes are characterized by a high content of non-polar and aromatic amino acids. On the other hand, the individual epitopes consist mainly of polar or charged amino acids. In contrast to the first group the control sera showed a low reactivity with these peptides.

Conclusion: We detect common IgE epitopes as the molecular equivalent of major allergens of α_{S1} -casein. Thus, the conclusion could be, that these epitopes should be destroyed in casein-based hydrolysates to provide the lowest allergenicity.

Universitäts-Kinderklinik, Mathildenstr. 1, D-79106 Freiburg/Brsg.

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5 FUNCTIONAL PROPERTIES OF THE PREDICTED SECOND NUCLEOTIDE BINDING FOLD OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR). C. Randak¹, E. A. Auerwald², H. Fritz², H.-B. Hadorn¹, I. Assfalg-Machleidt³, A. A. Roscher¹, W. Machleidt³

CFTR processing and mechanisms of channel activity regulation are topics of current interest in order to understand molecular pathology in cystic fibrosis. Electrophysiological data from Anderson and Welsh (Science 257, 1701), Smit et al. (Proc. Natl. Acad. Sci. USA 90, 9963) and Quinton and Reddy (Nature 360, 79) suggest that an interaction of ATP with the two nucleotide binding folds (NBFs) of CFTR contributes to channel opening and that ADP and AMP inhibit the channel with the ADP effect possibly mediated via NBF-2. In order to be able to study the interaction of nucleotides with CFTR-NBF-2 we have expressed this domain in *E. coli*, purified the soluble, natively folded fraction and characterized the recombinant protein by means of protein sequencing and circular dichroism

spectra. We could demonstrate specific binding of NBF2 to ATP-, ADP- and AMP-agarose, respectively. In order to quantify nucleotide binding of NBF-2 we have established nucleotide binding characteristics by measuring the fluorescence enhancement at 545 nm of trinitrophenylated (TNP labelled) nucleotides occurring when the excited (408 nm) TNP group is transferred into the less polar environment of a protein's nucleotide binding pocket that could be competed with non labelled nucleotides. All three adenine nucleotides were bound with high affinity (AMP > ATP > ADP) with K_d values ranging between 10 and 90 μ M. ATPase activity of NBF-2 couldn't be detected (detection limit 0, 11 mU). Our data clearly show that NBF-2 preferentially binds ATP and its hydrolysis products thus supporting the idea that this domain plays a crucial role in CFTR channel regulation regarding activation and inhibition in dependence on the cellular energy level.

¹Kinderklinik im Dr. von Haunerschen Kinderspital der Ludwig-Maximilians-Universität München, Lindwurmstr. 4, D-80337 München; ²Abteilung für Klinische Chemie und Klinische Biochemie der Chirurgischen Klinik Innenstadt der Ludwig-Maximilians-Universität München, Nußbaumstr. 20, D-80336 München; ³Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestr. 33, D-80336 München

6 LACTOSE-¹³C, ¹⁵N]UREIDE (LU)-BREATH-TESTS FOR INVESTIGATIONS IN THE HUMAN MICROBIAL FLORA. P. Leitzmann, K. D. Wutzke, W. Heine, C. Mohr, C. Plath, M. Radke, N. Eckhardt

Introduction: Diagnostic breath tests are to date almost exclusively directed to malfunctions of the stomach, liver, pancreas and the small bowel. Comparable tests for evaluation of the colonic microflora are currently not available. The main assumptions for the suitability of substrates to detect bacterial colonization are missing intestinal absorption, avoidance of fluctuation in osmolarity, and missing degradation by intestinal and intermediary enzymes.

Material and methods: One gram doubly-labeled LU corresponding to a ¹³C- and ¹⁵N-dose of 28.8 and 7.3 mg, respectively, was administered as a single-oral-pulse-labeling to seven adult volunteers. Breath and urine were collected at intervals of one and three hours, respectively, over a period of one day. ¹³C-enrichment in breath as well as ¹⁵N-enrichment in urinary urea and ammonia were measured by isotope ratio mass spectrometry.

Results: The ¹³C-enrichment of breath started at 4.5 hours reaching a peak value at eight hours after administration of LU. The urinary excretion rate of the ¹⁵N- and ¹³C-tracer was 33 and 15 % of the dose administered, respectively. The ¹⁵N-labeling of urinary urea started after five hours. Ammonia-enrichment was detectable between two and four hours. A total of 46 % of the ¹³C was found to be exhaled. Premedication of one gram unlabeled lastose ureide two hours prior to the pulse labeling resulted in an apparent shortening of the transit time to 2.5 hours. As there is evidence in vitro this phenomenon might be attributed to induction of the responsible enzyme activity of allantoinase amidohydrolase. Headspace analyses of bacterial cultures obtained from the feces of the subjects revealed a high ¹³CO₂-release from LU added to the culture medium in aerobic streptococci, Staphylococcus aureus, Proteus, Klebsiella, and Pseudomonas aeruginosa.

Conclusions: Stable labeled LU has proven to be a reliable marker for measuring microbial allantoinase amidohydrolase activity by means of a ¹³CO₂-breath-test procedure, and therefore in recording potential pathogenic aerobic intestinal bacteria. Disturbances of the microbial homeostasis caused by antibiotic and cytostatic therapy are considered to be assessable on this basis.

Dr. Peter Leitzmann, University of Rostock, Faculty of Medicine, Department of Pediatrics, Rembrandtstraße 16/17, Postfach 100888, D-18055 Rostock

7 DEVELOPMENT OF AN "ARTIFICIAL PRETERM INFANT" TO VALIDATE INDIRECT CALORIMETRY SYSTEMS. K. Bauer, C. Uhrig, A. Dieckmann, H. Versmold

During open flow-through indirect calorimetry to measure energy expenditure, O₂-consumption (VO₂) and CO₂-production (VCO₂) are calculated from concentrations in expired air sampled from a hood placed over the infant's head. Large dead space of the hood, low VO₂ and VCO₂ and/or low sampling flow may lead to incomplete gas sampling. Previous validations of indirect calorimetry systems by measuring the known VO₂

and VCO₂ of burning methanol could not detect incomplete gas sampling, because available burners produced a VO₂ and VCO₂ that is much higher than in preterm infants and cannot be used inside the patient hood.

Objective: To develop a methanol burning system that produces a VO₂ and VCO₂ as low as in preterm infants and that can be used to investigate influences of the infant's position and the geometry of the hood on the validity of the indirect calorimetry system.

Methods: We developed a pump-driven methanol burner mounted inside a life-size doll ("artificial preterm infant"). For the indirect calorimetry we used the Deltatrac II (Datex Corp, Finland).

Results: Our "artificial infant" produced a stable VO₂ (11.1 ml/min) and VCO₂ (7.4 ml/min) similar to a 1–1.5 kg infant. Using a hood for neonates (dead space 2500 ml) and a sampling flow of 3 l/kgmin, gas sampling was incomplete. VO₂ (–15 ± 5%) and VCO₂ (–14 ± 4%) were underestimated and changed markedly with different positions of the "artificial infant" inside the hood. These errors were eliminated by using a face mask (dead space 80 ml) (error VO₂ + 2.7%, error VCO₂ + 1.8%).

Conclusion: With the "artificial preterm infant" indirect calorimetry systems can be validated under real conditions. We found a marked underestimation of VO₂ and VCO₂ using a hood. Therefore a large number of published energy expenditure measurements in preterm infants performed in open flow-through systems with hoods need reevaluation.

Kinderklinik, Klinikum Benjamin Franklin, FU Berlin, D-12200 Berlin

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POSTNATAL DEVELOPMENT OF METABOLIC RATE IN PRETERM HUMAN AND SMALL MARSUPIAL NEONATES: DIFFERENT PATTERNS IN PATHOLOGICAL AND PHYSIOLOGICAL PREMATURITY*. D. Singer, E. Hehenkamp, U. Zeller[#], H. Schmidt[#], H.-J. Kuhn[#], W. Schröter

Background: Following a general biological rule, the specific basal metabolic rate is higher in small than in large organisms. As an exception, the neonate starts at a lower metabolic rate than would be expected from its small body size. The relatively rapid metabolic increase occurring in both term and preterm human neonates is usually regarded as a necessary consequence of the transition from intra- to extrauterine life. However, some biological observations seem to indicate that the "switching-on" of normal metabolic size relationship can be retarded in mammalian species which are physiologically born in a very immature state.

Objective: To compare the degree of metabolic reduction and the dynamics of metabolic increase under the conditions of "pathological" and "physiological" prematurity, metabolic measurements were done both in human preterm babies and in offspring of marsupial short-tailed opossum (*Monodelphis domestica*) which, by its birth weight of around 0.1 g (!), is one of the most impressive examples of physiological immaturity among mammalian neonates.

Methods: In human preterm neonates (30–32 weeks of gestation), O₂-consumption was determined by a DATEX DELTATRAC II Metabolic Monitor in the canopy (head-box) mode (indirect calorimetry). In the marsupial neonates, heat output was measured in a ThermoMetric 2277 Thermal Activity Monitor (direct calorimetry).

Results: In human preterm neonates, the O₂-consumption increases, within 20 days, from 5 to 9 ml · kg⁻¹ · min⁻¹, corresponding to a metabolic increase from about 70% to about 110% of the value to be expected from body size. In the marsupial neonates, no such increase is found in the heat output rate which, in this case, amounts to only 20% of predicted value. Nevertheless, the marsupial body weight undergoes an increase by nearly 500% in 10 days, whereas in humans, weight starts increasing only after the metabolic rate has reached its higher level.

Conclusions: Temporary metabolic deviation from the normal size relationship seems to be a general, possibly protective, feature of mammalian neonates. As compared with human preterm neonates, the metabolic rate is much more suppressed in the marsupial species and shows no tendency to increase in spite of continuous weight gain. These data suggest that the postnatal metabolic increase is not an unavoidable consequence of the transition from intra- to extrauterine life nor a prerequisite of extrauterine growth. However, in humans, unlike in especially adapted animals, the size-related metabolic rate seems to be "switched on" irrespective of neonatal immaturity. From this point of view, inadequate metabolic activation may play a primary role in various problems (such as growth retardation or respiratory distress syndrome) of the human preterm neonate.

Dept. of Pediatrics, Robert-Koch-Str. 40, [#]Dept. of Anatomy, D-37075 Göttingen

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SPONTANEOUS ALTERATIONS OF CEREBRAL BLOOD FLOW VELOCITIES IN HEALTHY TERM INFANTS. O. S. Ipsiroglu¹, S. Kuhle¹, E. Haxhija¹, M. Weninger¹, E. Michel², H. Pessenhofer³, A. Pollak¹

Alterations of cerebral blood flow velocities (cbfv) are associated with higher risk for cerebral morbidity in high risk neonates. Data on spontaneous cbfv alterations are scanty, because adequate equipment for long-term measurements in neonates was not available until recently*. We investigated the spontaneous alterations of cbfv in healthy term infants by transcranial (tc) – Doppler sonography, a novel application in neonates which allows long-term measurements.

Methods: A standard adult transcranial Doppler instrument was adapted in setting and energy output for neonatal measurements (DWL Multi-Dop X, buttonshaped 2 MHz probes (max. I_{SPTA} 3 mW/cm²), data recording possibility)**. Measurements: spontaneous cbfv alterations (syst., diast., mean cbfv velocities in both middle cerebral art.) were recorded in 11 healthy neonates without peripartur complications and related to behavioral state**. Three investigators measured 3 cbfv-tracings, each lasting at least 60 seconds. On-line heart rate, breathing movements, and oxygen saturation were recorded. Video tracings of the infant and the measurement setting were performed in order to estimate the behavioral state of the neonate (I, II, transient, III) and to detect technical or measurement artefacts. 195 tracings were first analysed visually using the criteria of cardiocardiography scores (stable baseline with/without alterations, variable baseline with intermittent continuous alterations) and then by spectral analysis.

Results: All infants showed beat to beat variability of cbfv, and additionally a slow variability of cbfv up to 1–6 cycles/min, within velocities altered up to 50%. We did not observe stable baselines of cbfv without alterations. Stable baselines with alterations were observed 37 times, variable baselines with intermittent alterations 42 times and variable baselines with continuous alterations 146 times. In addition to former investigators we also found alterations of derived indices, used to eliminate insonation angle errors (i.e. resistance index).

Conclusion: Our findings indicate a) that spontaneous alterations of cbfv are commonly found in healthy term infants, b) that the conventional way of data analysis (3–10 curves) cannot be accepted. Therefore with respect to cerebral morbidity, alterations of cbfv in high risk neonates have to be reevaluated with longer lasting Doppler tracings and related to behavioral state of the patient.

* Ipsiroglu OS, Steck J, Michel E, Hahn Th, Pessenhofer H, Jorch G, Pollak A: Is transcranial – (tc) – Doppler sonography safe for neonates? Pediatric Research 1994; 36; 1, 19A

** Prechtl HFR: The behavioral states of the newborn infant. Brain Res 1974; 76: 185–212

¹ Div. of Neonatology, Dept. Pediatrics, Univ. Hospital Vienna; ² Dept. Pediatrics, Berlin-Neukölln; ³ Dept. of Physiology, Univ. of Graz, Austria

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LIPOPEROXIDES IN TRACHEAL ASPIRATES AND DEVELOPMENT OF CHRONIC LUNG DISEASE IN NEONATES. L. Schrod, G. Frauendienst-Egger, M. Gerlich, H. Pfüller, H. B. von Stockhausen

Following histopathologic findings chronic lung disease of prematurity (BPD) is characterized by an exudative and a beginning proliferative phase one week after initial lung injury or prolonged artificial ventilation in very premature infants. To elaborate predictive markers in the first two weeks of ventilation for the development of BPD we studied several cellular and soluble parameters in 520 serially collected tracheal aspirates (TA) of 183 infants of various gestational ages. Airway inflammation with cell influx, increased free elastase activity and increased membrane permeability with higher plasma proteins like albumin in TA occurred in the most cases of long term ventilation and was not specific for the development of BPD. Premature infants with BPD had significant ($P < 0.05$) higher albumin concentrations in TA on day 5–10 compared to weight matched infants (<1500 g) without BPD but the individual predictive value remained low.

Liperoxide formation was measured by the malondialdehydethiobarbituric acid adduct with HPLC and spectrophotometric quantification. Malondialdehyde (MDA) was detectable in concentrations over 0.2 µmol/l only in 1 of 26 infants who underwent intubation for elective surgery but in all neonates with acute lung injury (respiratory distress syndrome, pneu-

monia) or long term ventilation over 48 hours. Excluding infants with bacterial colonization of the tracheobronchial tract, premature infants with BPD had significant higher MDA/albumin ratios than the weight matched control group between day 5 and 10 ($P < 0.02$). MDA values in TA did not correlate with MDA plasma concentrations. The data support the hypothesis that acute and chronic lung injury is mainly exerted by the local formation of oxygen radicals with subsequent structural alteration of membranes and other cell components. Although not specific for BPD, lipoperoxide formation might be a useful parameter for studies about the prevention and therapy of chronic lung disease during artificial ventilation in high risk infants.

Universitäts-Kinderklinik, Josef-Schneider-Str. 2, D-97080 Würzburg, FRG

11 NONINVASIVE ASSESSMENT OF ESSENTIAL FATTY ACID STATUS IN PRETERM INFANTS. U. von Schenck, B. Knoppke, B. Koletzko, D. Reinhardt

The supply and metabolism of essential fatty acids in preterm infants is receiving increased attention, because there are indications for a linkage to functional development. Traditionally, infantile fatty acid status has been assessed by analysis of plasma or red blood cell lipids. We attempted to estimate the essential fatty acid status from cheek cells to avoid the necessity of obtaining a blood sample.

Patients and methods: 21 preterm infants (mean gestational age 32.3 weeks, range 27–35 weeks) received breastmilk ($n = 6$), a preterm formula (PTF A, $n = 10$) without arachidonic (AA) and docosahexaenoic (DHA) acids or a formula with 0.44 % (of fatty acids) AA and 0.30 % DHA (PTF B, $n = 5$). Cheek cells were collected by gentle scraping with a cotton swap at hospital discharge. Lipids were extracted, phospholipids isolated by preparative thin layer chromatography, and fatty acid methyl esters analysed by high resolution gas-liquid chromatography. Results of groups were compared by one way analysis of variance.

Results: The method could be optimised to achieve reproducible detection of cheek cell phospholipid fatty acids. Results for AA and DHA reflected dietary intake and were lower in children fed PTF A.

Fatty acids in cheek cell phospholipids (% wt./wt., $M \pm SE$)

Diet:	Breastmilk:	PTF A:	PTF B:	P (ANOVA):
AA	3.33 ± 0.72	2.05 ± 0.18	3.83 ± 0.52	$= 0.022$
DHA	0.94 ± 0.29	0.25 ± 0.09	1.77 ± 0.32	< 0.001

Conclusions: 1. Analysis of cheek cell phospholipid composition allows estimation of infantile essential fatty acid status without obtaining a blood sample. 2. Cheek cell phospholipid composition reflects dietary lipid supply of preterm infants in early life.

Kinderpoliklinik d. Univ., Pettenkoferstraße 8a, D-80336 München

12 GASTRO-OESOPHAGEAL REFLUX (GOR): HOW MUCH DO WE MISS WITH pH-METRY? EVALUATION BY A COMBINED pH- AND IMPEDANCE-TECHNIQUE. H. Skopnik¹, J. Silny², O. Heiber^{1,2}, J. Schulz², G. Rau², G. Heimann^{1,2}

Conventional pH-metry detects acid GOR ($pH < 4$) exclusively. Because GOR associated symptoms are not caused by acid GOR only, usage of a pH independent method seems favourable. Therefore in this study pH-metry was simultaneously applied with a recently developed technique – intraluminal multiple electrical impedance procedure (IMP) – for measuring gastrointestinal motility. 17 infants below the age of 6 months with suspected GOR disease were investigated. Patients were studied during 2 feeding periods. The pH-electrode was placed 3 cm above the cardia, the six measuring segments for IMP reached from the cardia to the pharyngeal space. In all patients 185 acid episodes ($pH < 4$, > 15 sec.) were detected. In 103/185 episodes a unique pattern in the IMP-readings was found which indicated a retrograde oesophageal volume flow. These episodes were regarded as GOR episodes. 75/185 episodes occurred immediately after an acid GOR during the clearance process of the latter and 7/185 episodes could not be evaluated due to artefacts. The IMP pattern described as characteristic for a GOR was found in additional 493 episodes undetected by pH-metry. More than 75% of all GOR detected by IMP measurement reached the pharyngeal space. 53% of all GOR occurred during feeding and the 1st postprandial hour, 29% during the 2nd postprandial hour, and 18% during the remaining time until the next feeding. 69% of GOR during the first two postprandial hours and 12% of GOR during the remaining

time until the next feeding were detected by IMP measurement only, thus missed by pH-metry. The results show that IMP provides supplementary information to pH-metric data. IMP detects all GOR and seems to be especially useful to measure nonacid GOR. Therefore this technique is promising to improve the evaluation of unusual presentations of GOR such as recurrent aspiration, and to detect GOR in conditions with gastric hypoacidity.

Kinderklinik¹ und Helmholtz-Institut² der RWTH Aachen, Pauwelsstr., D-52057 Aachen

13 INHALED NITRIC OXIDE (NO) FOR SELECTIVE PULMONARY VASODILATION IN PEDIATRIC INTENSIVE CARE. S. Demirakça, C. Knothe, J. Bauer, J. Dötsch, P. G. Kühl.

Inhaled NO is increasingly used as a selective pulmonary vasodilator. This selectivity of NO has two major aspects: Macroselectivity leads to pure pulmonary vasodilation without any effect on systemic vasculature. Microselectivity causes preferential perfusion of ventilated areas, thereby improving gas exchange.

Objective: To evaluate dose response and long term effects of inhaled NO in children who could benefit from macro- or microselective pulmonary vasodilation.

Methods: ARDS patients with an Oxygenation Index (OI) > 20 cm H₂O/mmHg (microselectivity) or patients after cardiac surgery with a ratio of mean pulmonary to systemic arterial pressure (mPAP/mSAP) > 0.5 or after Fontan like operations with a central venous pressure (CVP) > 17 mmHg (macroselectivity) were included. After dose response testing with 5–80 ppm, NO inhalation was continued with the best effective dosage. Daily withdrawal attempts were made to monitor the need for long term inhalation.

Results: 25 patients were enrolled in this study. 11 ARDS patients and 14 patients after cardiac surgery (neonatal ARDS+PPHN $n = 6$ (I), pediatric ARDS $n = 5$ (II), pulmonary hypertension after corrective cardiac surgery $n = 8$ (E), Fontan like operations $n = 6$ (IV)). Best effective dosages were: group I = 20 ppm NO, II = 10 ppm NO, III + IV = 80 ppm NO. Within 24 h of NO inhalation OI improved in group I+II from 37 ± 13 to 14 ± 8 ($P = 0.008$). In group III mPAP/mSAP decreased from 0.76 ± 0.16 to 0.49 ± 0.08 ($P = 0.018$), in group IV CVP/mSAP decreased from 0.37 ± 0.15 to 0.25 ± 0.06 ($P = 0.043$) after 24 h NO inhalation. Duration of long term inhalation was 2–16 days. 24 of 25 patients survived.

Conclusions: Inhaled NO is an effective pulmonary vasodilator. The best effective microselective dose is 10 ppm. The macroselective effect increases with increasing doses up to 80 ppm. NO may improve outcome of diseases associated with pulmonary hypertension or ventilation perfusion mismatching.

S. Demirakça, Department of Pediatrics Justus-Liebig-University Giessen, Feulgen Str. 12, D-35383 Giessen

14 IMPROVEMENT OF PERIOPERATIVE HEMODYNAMICS AND OXYGEN STATUS BY NITRIC OXIDE (NO) INHALATION IN CHILDREN WITH CONGENITAL HEART DISEASE. J. Breuer, C. Irtel v. Brenndorff, M. Gass, W. Baden, L. Sieverding, J. Apitz

Objectives: It was shown recently, that the vascular endothelium releases NO, which causes relaxation of vascular smooth muscle cells. Therefore, it was evaluated whether continuous NO inhalation may reduce pulmonary artery pressure (PAP) and improve arterial oxygen saturation (SaO₂) in infants and children before or after surgery for congenital heart disease.

Methods: All patients ($n = 14$; age: 1 day–6.5 years) had secondary pulmonary hypertension (PH) or Acute Respiratory Distress Syndrome (ARDS) and were artificially ventilated with an inspiratory O₂ concentration of 100 %. NO was introduced into the afferent limb of the ventilator circuit, while continuously measuring the inspired NO concentration. After registration of all hemodynamic parameters during inhalation of 5, 10, 20 and 40 ppm NO, inhalation was continued with 1–30 ppm NO as required to obtain a stable hemodynamic situation. As soon as possible, the applied NO concentration was reduced and then discontinued.

Results: The hemodynamic condition and/or SaO₂ were significantly improved by NO in 13 patients (92%). SaO₂ increased from $80 \pm 3\%$ to $91 \pm 2\%$, due to a decreased intrapulmonary right-to-left shunt (32% vs. 22%). Mean PAP declined significantly from 37 ± 7 mmHg to 26 ± 5 mmHg, whereas mean systemic arterial pressure remained constant (54 ± 3 vs. 56 ± 4 mmHg). This was related to a selective reduction in pulmonary vascular resistance by $40 \pm 8\%$. NO therapy was applied with a median of

6 days. Regarding possible adverse effects of NO, methemoglobin concentration ($0.7 \pm 0.1\%$ vs. $1.5 \pm 0.2\%$) was only slightly increased which did not affect oxygen transport, and nitric dioxide (NO_2) formation was well below toxicologic relevant concentrations (e.g. 0.5 ppm NO_2 at 40 ppm NO).

Conclusions: Low dose NO inhalation selectively reduces pulmonary artery pressure and improves SaO_2 in children with congenital heart disease and PH or ARDS during perioperative care. The hemodynamic improvement seems to be related to an optimized right ventricular performance, since right ventricular afterload is reduced without changes in coronary perfusion pressure as often observed with other vasodilators.

Abt. Pädiatrische Kardiologie, Univ.-Kinderklinik, D-72076 Tübingen

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^{31}P MR SPECTROSCOPY: A USEFUL METHOD FOR EARLY DETECTION OF ABNORMAL MYOCARDIAL METABOLISM FOLLOWING ANTHRACYCLINE THERAPY. A. Eidenschink, H. Stern, G. Schröter, M. Nathrath, L. Stengel, S. Müller-Wehrich

Anthracyclines are effective and important cytotoxic drugs included in many chemotherapy regimens. Their most feared adverse effect is irreversible cardiomyopathy, which may occur up to years after cessation of therapy. With the aim of finding a sensitive method to early detect anthracycline-induced myocardial damage resulting in cardiomyopathy, we studied children without cardiac symptoms by ^{31}P magnetic resonance spectroscopy (MRS) after anthracycline therapy.

30 patients, mean age 14 years (3.5–26 years), were examined by MRS 3.25 ± 3.5 years after anthracycline therapy; 13 had ALL, 2 AML, 5 NHL, 1 Hodgkin's disease, 3 osteosarcoma, 1 Ewing's sarcoma, 2 nephroblastoma and 3 rhabdomyosarcoma. The mean total dose of anthracyclines was 300 mg/m^2 (60–750 mg/m^2). All patients had normal results of ECG and 2D-echocardiography on repeated examinations. As controls served 5 patients before cytotoxic therapy and 16 healthy volunteers, mean age 19 years (7–29 years). ^{31}P MR spectra of anteroapical myocardium were obtained at 1.5 Tesla; the selected myocardial volume was $50 \pm 18 \text{ ml}$. The peak areas of phosphocreatine (PCr), phosphodiester (PDE) and adenosine triphosphate (ATP) were calculated.

The PCr/ATP ratio in the patients treated with anthracyclines was 0.86 ± 0.35 , which was significantly lower than in the control group (1.22 ± 0.35). The PDE/ATP ratio was 0.98 ± 0.62 in the patients and 0.82 ± 0.37 in the controls.

With higher cumulative doses of anthracyclines and after a longer follow-up period, PCr decreases in relation to ATP.

^{31}P MRS is a non-invasive method for the early detection of abnormal myocardial metabolism after anthracycline therapy. Identification of patients at risk for anthracycline-induced cardiomyopathy early during chemotherapy may be helpful in modifying the chemotherapeutic regimen in order to avoid this potentially lethal complication.

Children's Hospital of the Technical University, Kölner Pl. 1, D-80804 Munich

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OPTIC NERVE SONOGRAPHY AND INCREASED INTRACRANIAL PRESSURE. A NEW QUANTITATIVE ANALYSIS. K. Helmke, H. C. Hansen*

Both, early detection and quantification of increased intracranial pressure (ICP) can be decisive for effective therapy. As of yet, ICP-measurement is an invasive procedure.

By sonography of the orbits the Optic Nerve Sheath Diameter (ONSD) can be exactly defined. The optic nerves, like other parts of the central nervous system, are surrounded by cerebrospinal fluid (CSF) and the meninges. Increase of ICP can either be associated with swelling of the optic nerve and/or by enlargement of the perineural subarachnoidal compartment by passive CSF-inflow.

We were able to prove this phenomenon by *in vitro* experiments (post mortem optic nerve preparations) and clinically in patients. Normal data were established in 118 healthy control persons between the age of 0 and 87 years. The following ONSD values are to be considered as pathological: children up to the age of 4 years >3.5 to 4 mm ($n = 25$), children above the age of 5 years as well as adults >4.5 to 5 mm ($n = 26$).

In the last two years, a total of 24 children and 32 adults suffering from an increase in intracranial pressure have thus been examined. The sonographic measurements were compared with the findings of other methods (epidural pressure probe, CCT, transcranial ultrasound). As a result of this comparison it can be ascertained that the initial increase of ICP leads to an immediate expansion of the ONSD. A drop in ONSD could be noted

round about 12 hours after ICP had decreased. Sonographic ONSD-measurement has been established in our institution as a clinically relevant tool for noninvasive ICP-monitoring.

Dept. of Pediatric Radiology, Children Hospital; Neurological Clinic (*); University Hospital Hamburg-Eppendorf; Martinistr. 52, D-20246 Hamburg, Germany

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REGULATION OF FUROSEMIDE-SENSITIVE ION TRANSPORT BY HYDROXYEICOSATETRAENOIC ACIDS. A. Köckerling, M. Wegmann, S. Reinalter, H. W. Seyberth

In hyperprostaglandin E_2 syndrome impairment of furosemide-sensitive Cl^- reabsorption seems to be the major mechanism causing renal loss of salt and water. Suppression of elevated PGE_2 levels by indomethacin improves fractional Cl^- reabsorption and reduces polyuria as well as hypokalaemia. Inhibition of cyclooxygenase, however, provides only partial success. Further metabolites of arachidonic acid may play an additional role in tubular transport alteration. 20-hydroxyeicosatetraenoic acid (20-HETE) and 12(R,S)-HETE – both derived from the cytochrome P450 pathway – have been supposed to affect epithelial ion transport in a furosemide- and ouabain-like manner, respectively. We tested this hypothesis using furosemide-sensitive intestinal Cl^- secretion as a model resembling tubular transport in the thick ascending loop of Henle. As in the kidney, it depends on several cellular mechanisms including $\text{Na}^+2\text{Cl}^- \text{K}^+$ cotransport, Na^+K^+ -ATPase, opening of Cl^- channels by increased cAMP and K^+ recycling via a specific conductivity. Rat colonic segments were mounted in Ussing chambers and electrogenic ion transport was measured as short circuit current (I_{sc}). 20-HETE up to $3 \times 10^{-6} \text{ M}$ had no effect on I_{sc} , neither in secreting nor in absorbing tissues, indicating that this metabolite is not involved in the regulation of furosemide- or ouabain-sensitive ion transport. 12(R,S)-HETE ($3 \times 10^{-7} \text{ M}$) significantly inhibited Cl^- secretion after stimulation with PGE_2 , theophylline or DB-cAMP by 33%, 24% and 30%, respectively. In contrast furosemide-sensitive K^+ secretion induced by epinephrine was not affected. 12(R,S)-HETE had also no effect on ouabain-sensitive Na^+ absorption stimulated by aldosterone. Thus this cytochrome P450 metabolite acts on a site different from both, $\text{Na}^+2\text{Cl}^- \text{K}^+$ cotransport and Na^+K^+ -ATPase. Since 12(R,S)-HETE had no influence on total epithelial conductance, control of apical Cl^- channels is unlikely, too. We conclude, that 12(R,S)-HETE, but not 20-HETE, inhibits furosemide-sensitive epithelial Cl^- transport. The underlying mechanism, however, still remains unexplained.

It may be involved in the pathogenesis of hyperprostaglandin E_2 syndrome.

Universitäts-Kinderklinik, Deutschhausstr. 12, D-35033 Marburg, Germany

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ASSESSMENT OF FLOW-VOLUME CURVES BY THE ISOFLOW TECHNIQUE IN HEALTHY CHILDREN AND IN CHILDREN WITH OBSTRUCTIVE LUNG DISEASE. A. Schibler, R. Kraemer

The main goal of ambulatory lung function testing performed in patients with asthma, obstructive lung disease and cystic fibrosis (CF) is to evaluate the degree of airway obstruction. In children the ability to cooperate is rather limited. In consequence lung function devices should be cooperation dependent less than possible. For that purpose a apparatus, featuring maximal but appropriate information reflecting the underlying physiology, was developed. Using constant expiratory flows at different flow levels (1- and 2-liter-isoflows, IFP1 and IFP2) the effort independent part of the flow volume curve may be determined. In 104 healthy children, 39 asthmatic children and 22 patients suffering from CF such isoflow measurements in comparison with peakflow measurement obtained by the Wright Peakflow Meter (PF) were recorded and evaluated in relation with standard lung function parameters such as FEV1, Peakflow (PEF), MEF50, MEF25. Good correlations were found between the above mentioned parameters and the IFP1 and IFP2 (r between 0.71 and 0.94, $P < 0.001$). However, using the statistical approach proposed by Bland, agreement between FEV1 and PF had to be considered as poor, where as good relationship between FEV1 and IFP1 and IFP2 is demonstrated. Therefore, the isoflow technique may be used as a good alternative for lung function measurements as home monitoring. In addition most patients preferred isoflow measurements, because of less side effects (effort induced coughing) than during peakflow measurements.

Division of Paediatric Respiratory Medicine, Departement of Paediatrics, University of Berne, Inselspital, CH-3010 Berne, Switzerland

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GUANIDINOACETATE-METHYLTRANSFERASE DEFICIENCY: FIRST REPORT ON A PATIENT WITH AN ENZYME DEFECT IN CREATINE BIOSYNTHESIS. S. Stöckler¹, F. Hanefeld¹, J. Frahm², B. Schmidt³, K. v. Figura³

In a male infant with severe statomotor retardation and progressive extrapyramidal movement disorder, *in vivo* proton and phosphorus magnetic resonance (MR) spectroscopy revealed a depletion of cerebral creatine/creatine phosphate and an accumulation of guanidinoacetate, suggesting an enzyme defect in creatine biosynthesis at the level of guanidinoacetate methyltransferase (GAMT) (1).

GAMT activity was determined in liver extracts from the patient and from 3 controls by measurement of the transfer of ³H labelled methyl group from S-adenosylmethionine to guanidinoacetate resulting in ³H labelled creatine which was separated from S-adenosylmethionine by cation exchange chromatography on HPLC. GAMT activity was found to be severely defective in this patient.

Oral substitution of 4 g creatine monohydrate daily over a period of 11 months resulted in a striking improvement of the extrapyramidal movement disorder and in a considerable progress of statomotor development. The EEG turned from a very slow background (1.5–3/s) and multifocal spike wave activity to a regular theta activity, and bilateral lesions of the globus pallidus on T1 and T2 weighted MR images disappeared. Extremely low plasma creatinine concentrations (0.08 mg/dL; normal: 0.2–0.4) and low urinary creatinine excretion (1.4 mg/kg/d; normal: 8–20) normalized after 3 months of therapy (0.4mg/dL and 16.6 mg/kg/d) and remained at the same normal values during the further course. Brain creatine increased to 50–60 % of normal values and there was a parallel increase of creatine phosphate.

GAMT deficiency is a new, potentially treatable disorder of creatine biosynthesis. The incomplete restoration of creatine/creatine phosphate in the brain suggests different accessibilities of exogenous creatine to the brain. Early treatment might largely prevent brain injury. Methods for screening and non invasive biochemical diagnosis are needed in future.

* S Stöckler et al. Creatine deficiency in the brain: A new, treatable inborn error of metabolism. *Pediatr Res* 1994; 36: 409–413

Kinderklinik/Neuropädiatrie¹, Zentrum Biochemie, Abteilung Biochemie II², Universität Göttingen und Biomedizinische NMR Forschungs-GmbH, Max-Planck-Institut für biophysikalische Chemie Göttingen²

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ALTERED SORTING OF LYSOSOMAL MEMBRANE PROTEINS IN GAUCHER HEPATOCYTES WITH THE G202R MUTATION. K. P. Zimmer, P. le Coutre, H. Aerts, J. S. O'Brien, K. Harzer, M. Fukuda, T. Deufel, K. Ullrich, E. Harms

Gaucher disease is characterised by a marked phenotypic heterogeneity which is still unexplained in spite of progress made in the analysis of the mutations in the glucocerebrosidase gene. Investigating the role of cell biological processes as modifying factors in the pathogenesis of the disease, we examined whether intracellular transport of the lysosomal proteins, saposin C and cathepsin D, as well as the lysosome-associated membrane glycoproteins, lamp-1 and lamp-2, was altered in hepatocytes of a patient with an acute neuronopathic form of Gaucher disease. These proteins employ different intracellular sorting mechanisms for their transport to lysosomes. Lamp-1 and lamp-2 possess a tyrosine residue in their cytoplasmic tail. In contrast, saposin C and cathepsin D are soluble proteins and are sorted to lysosomes by a mannose 6-phosphate receptor-dependent mechanism.

The mutation was identified by PCR and subsequent SSCP analysis followed by DNA sequencing of cloned PCR products. Western blot analysis was performed to determine the amount of cross-reactive protein in the patient's fibroblasts. Using ultrathin frozen sections as well as poly- and monoclonal antibodies, we determined the intracellular localisation of saposin C, cathepsin D, lamp-1 and lamp-2 in liver and fibroblasts of our patient. The cellular binding sites were visualised by immunogold in the electron microscope.

The patient was found to be homozygous for a G-to-A substitution at nucleotide 721 resulting in the replacement of a glycine by an arginine (G202R). The amount of cross reactive material was increased in the fibroblasts of our patient in contrast to the genotype L444P, the most prevalent mutation in patients with the neuronopathic form. The intracellular amounts of all four lysosomal proteins were enhanced in the affected hepatocytes and fibroblasts. Saposin C, cathepsin D as well as lamp-1 and lamp-2 were found adjacent to the twisted tubules containing glucosylceramide. Lamp-1 and lamp-2 were present on the basal and apical mem-

brane of diseased hepatocytes, a finding that was absent in affected fibroblasts, control hepatocytes as well control fibroblasts. In contrast to lamp-1 and lamp-2, the amounts of saposin C and cathepsin D were not significantly increased on the cell surface of the patient's hepatocytes.

We conclude that the intracellular targeting of lamp-1 and lamp-2 was altered possibly due to saturation of the sorting signal in glucosylceramide accumulating tissue cells of our patient. In contrast to saposin C and cathepsin D, the routing of lamp-1 and lamp-2 to lysosomes occurred via the cell surface.

Universitätskinderklinik, Albert-Schweitzer-Straße 33, D-48149 Münster

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TWO PATIENTS WITH A GENERALIZED PEROXISOMAL DISORDER BUT ONLY MINIMALLY IMPAIRED DE NOVO BIOSYNTHESIS OF PLASMALOGENS. A CLUE TO THE BIOGENESIS OF PEROXISOMAL MEMBRANE STRUCTURES? W. Erwa, K. Fumic, B. Flies, G. S. Stöckler, E. Paschke

Disorders of peroxisomal biogenesis are characterized by a loss of multiple peroxisomal functions including the peroxisomal steps in the biosynthesis of plasmalogens and general lack of ultrastructurally or immunohistochemically recognizable peroxisomes in various organs and tissues. Despite rather uniform biochemical findings, at least 3 different clinical entities, *Zellweger syndrome*, *Neonatal Adrenoleukodystrophy* and *Infantile Refsum disease* can be distinguished by the age of onset and the degree of clinical illness.

We now report on two unrelated female patients with typical clinical signs of a disorder of peroxisomal biogenesis, like severe hypotonia, liver dysfunction and typical facial traits of Zellweger syndrome. Many typical biochemical features like elevated concentration of very long chain fatty acids and phytanic acid in serum and of pipecolic acid in urine; >90% of soluble catalase activity in cultured fibroblasts; lack of structures resembling peroxisomes upon immunofluorescence staining with an antibody against human catalase and severely decreased oxidation rate of [¹⁴C]-lignoceric acid in fibroblasts were present.

Plasmalogen biosynthesis in fibroblasts however, measured by the ratio of incorporation of [¹⁴C]-hexadecanol and 1-O-[³H]-hexadecyl-sn-glycerol into the alkenyl moiety of plasmalogens, was only slightly above the control values (1.1 and 0.9 vs. 0.7–0.9 for controls; NALD patients: 2.5–5.9; Zellweger patients: >5). Somatic cell fusion experiments showed genetic complementation with all but one cell line, which in a previous paper had been characterized as being different from other disorders of peroxisome biogenesis (Roscher et al.; *Ped. Res.* 26, 67–72, 1989). Since ether lipid synthesis was in the range of NALD in fibroblasts of this former patient, only a partial genetic correlation to our cases can be assumed. Finally, preliminary experiments provided evidence that dihydroxy-acetonephosphate acyltransferase (EC 2.3.1.42) was sedimentable at 9500 g and thus associated with a membrane structure. Characterization of these membranes by subcellular fraction studies are currently performed to reveal further data on the so far unknown relation between the biogenesis of peroxisomal membrane structures and the de novo formation of ether lipids.

Universitäts-Kinderklinik Graz, Auenbruggerplatz 30, A-8036 Graz

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ACHONDROGENESIS TYPE IB IS ASSOCIATED WITH A DEFECT IN THE METABOLIC ACTIVATION OF SULFATE. A. Superti-Furga¹, H. J. v. d. Harten², D. H. Cohn³, D. L. Rimoïn³, B. Steinmann¹, R. Gitzelmann¹

Background: We have recently described a patient with achondrogenesis type IB, an autosomal recessive and lethal chondrodysplasia, whose cartilage contained drastically reduced amounts of sulfated proteoglycans. His fibroblasts failed to incorporate ³⁵S-sulfate into macromolecules as a consequence of their inability to synthesize the sulfate donor, 5'-phosphoadenosine-3'-phosphosulfate (PAPS) (A. Superti-Furga, *Am J Hum Genet* 55: A344, 1994).

Methods: To test for the sulfate activation defect in this and other patients, we have studied fibroblast or chondrocyte cultures of four patients with histology-proven achondrogenesis type IB, two with type IA, one with type II, and several controls, by labelling with ³⁵S-sulfate, with or without ³H-glycine, followed by liquid scintillation counting, SDS-PAGE of proteins and proteoglycans, and separation by paper electrophoresis of ³⁵S-labelled free sulfate, APS, PAPS, and sulfated macromolecules.

Results: All four achondrogenesis type IB cell cultures (but none of type IA or II cultures) showed (1) low ³⁵S/³H-ratios in macromolecules after

double-labelling, (2) absence of sulfated proteoglycans, and (3) normal intracellular levels of ^{35}S -labelled free sulfate but markedly reduced levels of intracellular APS and PAPS.

Conclusions: Achondrogenesis type IB is associated with a defect in the metabolic activation of sulfate which leads to insufficient sulfation of proteoglycans and other macromolecules. Expression of the defect in fibroblasts as well as in chondrocytes allows a new diagnostic access to the disorder.

¹Dept. of Pediatrics, University of Zurich, Steinwiesstr.75, CH-8032 Zürich; ²Free University Hospital, Amsterdam; ³Cedars-Sinai Medical Center, Los Angeles

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MEROSINOPATHY: CLINICAL SPECTRUM AND CORRELATION TO IMMUNOCYTOCHEMISTRY. V. Straub, R. Herrmann, K. Meyer, T. Kahn, M. Wagner, F. Hanefeld, K. Arahata, F. M. S. Tome, H. G. Lenard, T. Voit

Merosin, the heavy chain component of the heterotrimer muscle laminin, is one of the major extracellular matrix proteins present in basement membranes. Lack of merosin has recently been detected as the likely cause of one form of congenital muscular dystrophy (CMD). Affected patients typically show hypotonia of muscular origin at birth or in the first month of life, early contractures, markedly elevated serum CK levels, and dysmyelination of the central nervous system. Mode of inheritance is autosomal recessive, and the merosin (or laminin M chain, LAMM) gene locus has just been mapped to chromosome 6q22-q23. We present 11 children with CMD who showed merosin deficiency in their skeletal muscle using immunofluorescence microscopy. The clinical spectrum and the course of the disease were heterogeneous. Complete lack of merosin expression was demonstrated in 9 patients. None of these patients became ambulant. The most severe phenotype displayed severe congenital hypotonia and arthrogryposis. All of them exhibited imaging changes in the white matter on magnetic resonance images (MRI) due to dysmyelination. Immunofluorescent staining of skeletal muscle cryosections revealed compensatory overexpression of the laminin A chain, which is not expressed in normal mature muscle basement membranes. In contrast to complete merosin deficiency two patients learned walking around the age of 18 month and 3 years. Both showed reduced but preserved merosin expression in the majority of their fibers. Preliminary mRNA studies using polymerase chain reaction (rt-PCR) so far failed to detect larger deletions or splice mutations of the merosin gene in four patients. Because low abundance of deletion mutation may hamper potential diagnosis in the future we developed a direct approach for prenatal assessment by merosin staining of chorionic villous samples of human placenta.

Department of Pediatrics and Pediatric Neurology, Heinrich Heine-University Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany

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HUMAN SEVERE COMBINED IMMUNODEFICIENCY (SCID) CAUSED BY RECOMBINASE DEFICIENCY. K. Schwarz^{1,2}, L. Ludwig¹, D. Lindner¹, W. Friedrich², R. Seger³, T. E. Hansen-Hagge¹, E. Kleihauer², C. R. Bartram^{1,2}

Human SCID is a heterogeneous disease and well suited to get information about differentiation and activation processes in lymphocytes. Our objective was, to analyse SCID patients and their families for mutations on RAG1 and RAG2, some of the earliest presently known developmental keys in lymphocyte ontogeny, genes, which control or interact directly in the V(D)J rearrangement of Ig or TCR receptor genes.

30 unselected SCID patients were analysed at the DNA level at the RAG loci for single stranded conformation polymorphism (SSCP), indicating possible mutations. The mutations were confirmed by sequencing and DNA analyses of the families were conducted to proof heritage.

Four children in four families were identified with a RAG1 deficiency, three children in two families with RAG2 mutations, with one male patient having a RAG1 and RAG2 mutation simultaneously. One deletion, missense and nonsense mutations were detected. The functional consequences of the mutations in the RAG genes were tested in an *in vitro* recombination assay.

We conclude, that at least 20% of SCID patients were defective at the RAG genes.

The phenotype of the patients is very variable; the common denominator is the absence of B cells, with (low) T cells present or absent. The T cells

originate either from the mother by maternofetal transfusion or when low in number possibly due to a 'leaky' phenotype of the mutation.

¹Section of Molecular Biology, ²Pediatrics II, University of Ulm, Ulm, Germany, ³Department of Pediatrics, University of Zürich, Zürich, Switzerland

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ANALYSIS OF THE p53 GENE AND THE p21 GENE IN CHILDHOOD MEDULLOBLASTOMAS. W. Scheurle¹, N. Sörensen²

Mutations of the p53 gene are the most common genetic event in human cancer. The tumor suppressor p53 and its downstream effector p21, an inhibitor of cyclin-dependent kinases, play an important role in the regulation of the cell cycle. In case of DNA damage induction of the p53/p21 pathway may lead to G1 arrest and DNA repair or may result in apoptosis if DNA damage is irreparable.

We screened 15 childhood medulloblastomas for mutations in the hot spot regions of the p53 gene using the highly sensitive single strand conformational polymorphism (SSCP) analysis of polymerase chain reaction-(PCR)-amplified DNA. A point mutation was found in only one tumor, suggesting that mutations of the p53-gene are not prominent features in medulloblastoma tumorigenesis. Searching for alternative mechanisms how this important pathway might be disrupted we analyzed 10 medulloblastomas for deletions or rearrangements of the p21 gene. Tumor DNA and control DNA were analyzed by duplex-PCR analysis and Southern blot analysis. Tumor specific deletions and rearrangements of the p21 gene were found in 4 out of 10 medulloblastomas. This is the first report of p21 deletions/rearrangements in human tumors. We suggest that inactivation of the p53 downstream effector p21 is functionally equivalent to p53-mutations and that mutations of the p21 gene may play an important role in medulloblastoma tumorigenesis. Moreover our findings bear therapeutic implications since an intact p53/p21 dependent apoptosis pathway is essential for effective radiotherapy or chemotherapy with etoposides.

Depts. of Pediatrics¹ and Pediatric Neurosurgery², University of Würzburg; D-97080 Würzburg

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INTRACELLULAR KINETICS OF CYTOSINE-ARABINOSIDE-TRIPHOSPHATE IN LEUKEMIC BLAST CELLS FROM CHILDREN. J. Boos, M. Schiller, B. Hohenlöcher, P. Schulze-Westhoff, J. Ritter, H. Jürgens

Cellular uptake and intracellular phosphorylation to the nucleotide cytosine-arabinoside-triphosphate (Ara-CTP) is the precondition for the cytostatic effect of cytarabine. The pharmacokinetics of Ara-CTP in leukemic cells was reported to be of clinical importance in adult nonlymphoblastic leukemia. Therefore, a monitoring of cellular pharmacokinetics in pediatric leukemia *in vivo* and *in vitro* was initiated in order to contribute to optimization of Ara-C treatment schedules.

Separated peripheral or bone marrow blast cells from 66 children with ALL (51) and AML (15) were incubated in Ara-C containing medium (1 h; 1 or 3 µg/ml) followed by reincubation in Ara-C free medium (3 h). Retention at the end of the second incubation period was expressed in % of the 1 h level. In 8 children (7 AML, 1 ALL) the Ara-CTP concentrations in circulating blasts were monitored during induction therapy *in vivo*.

In vivo the mean intracellular steady state concentration during continuous infusion (100 mg/m²/24 h) was 70 ± 69 pmol/10⁷ cells. These cellular levels and single observations following short term infusion corresponded to the pharmacokinetic parameters observed in the *in vitro* model system.

In vitro the Ara-CTP accumulation (1 h) did not show marked differences between AML, T-ALL and non-TALL. The Ara-CTP retention, however was significantly lower in AML (34 ± 18%, $P < 0.0006$) and T-ALL (37 ± 15% $n = 8$ $P < 0.002$) compared to non-T-ALL (67 ± 25% $n = 33$) at initial diagnosis. Lower Ara-CTP retentions were observed in relapsed leukemias (non-T-ALL 51 ± 16%, $n = 14$) but up to now the difference is not significant ($P < 0.007$).

The Ara-CTP retention declined with the risk groups defined by the ALL-BFM-protocol (SRG 79 ± 29, MRG 59 ± 25, HRG 47 ± 21). Patients with persistence of peripheral blast cells on day 8 of treatment with prednisone showed a lower Ara-CTP retention compared to the others ($P < 0.03$). Complete bone marrow remission on day 15 was correlated with significantly higher Ara-CTP retention (longer intracellular half-life, $P < 0.03$).

These differences in cellular Ara-CTP retention – not formation – comprise a pharmacokinetic rationale for continuous Ara-C infusion especially in AML and T-ALL as an alternative to the intensification by high-dose Ara-C schedules.

Department of Pediatric Hematology & Oncology, University of Münster, Albert-Schweitzer-Str. 33, D-48159 Münster, FRG

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MONOAMINE TRANSPORTER GENE EXPRESSION IN NEUROBLASTOMA CELL LINES: CORRELATIONS TO MIBG UPTAKE AND TYROSINE HYDROXYLASE GENE EXPRESSION. H. N. Lode, G. Bruchelt, G. Seitz, S. Gebhardt, J. Beck, D. Niethammer

Radiolabelled meta-iodobenzylguanidine (mIBG) has been widely used in therapy of children with advanced stage neuroblastoma but still with poor results in long term survival, which is possibly due to insufficient tumor uptake. Recently it was demonstrated that mIBG can be taken up by the noradrenaline transporter with all characteristics of the specific monoamine transport system Uptake 1. mIBG uptake inhibition experiments in SK-N-SH cells suggest also evidence of the presence of dopamine- and serotonin-transporters. The aim of this study was to investigate which of these transporters are involved in mIBG uptake of neuroblastoma cells. Tyrosine hydroxylase gene expression, the key regulatory enzyme of catecholamine synthesis, was evaluated since a coordinated regulation of catecholamine transporters and tyrosine hydroxylase was expected. Therefore, noradrenaline, dopamine and serotonin transporter gene expression was measured in 6 different neuroblastoma cell lines using a semi-quantitative RT-PCR approach with glyceraldehyde-3-phosphate dehydrogenase as internal standard. Parallel, mIBG uptake was determined. Despite a significant mIBG uptake inhibition by dopamine and serotonin, neither dopamine- nor serotonin-transporter gene expression was observed in any neuroblastoma cell line investigated. Noradrenaline transporter gene expression was found in 4 of 6 cell lines and correlated with specific mIBG uptake. Noradrenaline transporter gene expression was found to be inversely correlated to tyrosine hydroxylase gene expression. This study shows that the noradrenaline transporter is the major mIBG uptake system in neuroblastoma cells and its inverse coexpression with tyrosine hydroxylase suggests a regulation via intracellular catecholamine levels. Therefore catecholamine synthesis inhibitors are possible stimulators of noradrenaline transporter gene expression.

Children's Hospital, Universitäts-Kinderklinik, Rümelinstraße 23, D-72070 Tübingen

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PROGNOSTIC VALUE OF FOS, JUN AND RAS – ONCOGENE EXPRESSION IN NEWLY DIAGNOSED CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA. A. Sauerbrey, M. Volm, G. Stammler, F. Zintl

In a retrospective study the expression of the oncogenes c-fos, c-jun and c-pas-ras was analyzed at the protein level in newly diagnosed cases of acute lymphoblastic leukemia (ALL) in children. In addition, the results were associated with the clinical outcome after treatment with cytostatic agents.

Blast cells obtained from 104 children with untreated ALL were determined by the streptavidin-biotin-peroxidase complex method and specific antibodies. The age distribution of the patients (46 male, 58 female) included 3 patients younger than one year, 77 younger than 10 years and 24 older than 10 years. According to their immunological subtypes the patients were grouped in 17 pre-B-ALL, 47 c-ALL and 33 T-ALL. The leukemic cells from a selection of 20 representative patients were investigated for the c-fos-mRNA expression by RT-PCR. All patients investigated in this study received therapy according to a modified BFM-protocol.

Of the 104 cases 52 were positive for Fos (50%), 65 for Jun (63%) and 22 for Ras (21%). The expression of the oncoproteins was independent of clinical characteristics like age, sex, immunological subtype and the initial peripheral blast cell count (PBC). The investigation of relationships between oncoprotein-expression and response to chemotherapy showed that Fos-positive patients had a significant higher relapse rate ($P = 0.0002$). A similar trend was found for Jun-positive cases ($P = 0.073$). In contrast, the expression of Ras showed no significant correlation with the relapse rate ($P = 0.98$).

Corresponding results were obtained for the relapse free interval. The probability of continuous first remission was significantly lower in patients with Fos- and Jun-positive blast cells (Fos: $P < 0.001$; Jun: $P = 0.09$; Ras: $P = \text{NS}$, log-rank test). In multivariate analyses (Cox regression model) included Fos-expression and initial PBC we could demonstrate that Fos is a significant prognostic factor for the probability of continuous first remission (Fos: $P = 0.032$, PBC: $P = 0.007$). In order to substantiate the significant correlation of c-fos-protein expression with the relapse rate the c-fos-mRNA expression was investigated in a collective of 20 patients by semiquantitative RT-PCR. The PCR-assay and immunocytochemistry corresponded in 14 of 20 cases (70%). The results of the PCR-assay demonstrated also a trend for increased c-fos-mRNA expression and higher relapse rate. The results of the present study reveal a connection between clinical drug resistance and the expression of Fos- and Jun-oncoproteins in blast cells of children with ALL. The Fos-oncoprotein is a prognostic predictor for the probability of continuous first remission in these patients.

University of Jena, Department of Pediatrics, Kochstr. 2, D-07740 Jena, Germany

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MOLECULAR CHANGES IN MYELODYSPLASTIC SYNDROMES. A. S. Schulz, L. Ludwig, H. Kessler, J. W. G. Janssen, E. Kleihauer, C. R. Bartram

Myelodysplastic Syndromes (MDS) reflect in their different subgroups distinct stages in leukemogenesis preceding overt acute myelocytic leukemia. Therefore, MDS offer a unique possibility to study the multistep process of malignant transformation of hematopoietic cells *in vivo*. In adult as well as in childhood patients bone marrow cells are of clonal origin and possibly hematopoietic stem cells are involved as suggested by X-chromosome inactivation studies. Mutations of the RAS and FMS oncogenes have been described in about 59% and 27% of patients, respectively. On the other hand, mutations of the p53 and neurofibromatosis type 1 (NF-1) tumor suppressor genes are rare in MDS. Since rearrangements of the ALL1/MLL gene on chromosome 11q23 are common in infants with acute leukemia, we looked for ALL1 rearrangements in MDS patients. In only one child out of 47 patients with primary MDS, who developed acute myeloblastic leukemia, an ALL1 rearrangement was found. Since deletions of the cyclin-dependent kinase inhibitor p16 represent the most frequent genetic lesion in human malignancies including acute lymphoblastic leukemias, we performed Southern Blot analysis in 45 MDS patients and observed a p16 germline status in all cases. Thus, p16 mutations may represent a critical step in the development of overt leukemia beyond the preleukemic stages represented by MDS.

Section of Molecular Biology, Department of Paediatrics II, University of Ulm, Helmholtzstrasse 10, D-89081 Ulm, Germany

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LIVER GROWTH AND PERFUSION AFTER SEGMENTAL LIVER TRANSPLANTATION IN CHILDREN. B. Rodeck, P. F. Hoyer, M. Scherbring, M. Melter, B. Ringe

The technique of segmental liver transplantation (sLTx) opened a way out to overcome the shortage of suitable livers for small recipients. Patient survival rates are parallel to those obtained with whole liver transplantation (wLTx). For long term rehabilitation adaptive liver growth and adequate perfusion is crucial, however, morphometric and hemodynamic parameters in growing children with sLTx are not available. Seventeen children who received a sLTx and 25 with a wLTx who had follow-up controls 1 and 2 years after LTx were studied. Mean age at time of transplantation was 4.3 ± 3.5 years in sLTx and 10.3 ± 6.0 in wLTx, mean height 98 ± 21 cm and 122 ± 30 . At follow-up control liver enzymes, bilirubin and prothrombin time were in the normal range in both groups. Liver dimensions were measured by ultrasound in the axillar, medioclavicular and sternal line, and hemodynamic parameter by Doppler sonography in the portal vein and art. hepatica using an Acuson 128 machine. Maximal (Vmax), minimal (Vmin) and time average velocity (TAV) was measured and the resistance index (RI) calculated.

Results: One and two years after LTx liver dimensions were at a mean in the upper normal range of healthy controls. In a longitudinal study over 4 years, a steady growth parallel to normal percentiles was observed, irrespectively of sLTx and wLTx. Spleen size was above the normal range and did not show any tendency of regression. Mean Vmax in the art. hepatica in sLTx and wLTx was 48 cm/sec versus 28 after one year and 30 versus 35 after two years, Vmin 16 versus 14 and 11 versus 13, the RI 0.66 versus 0.55 and 0.59 versus 0.73 (P for all parameters > 0.05). Maximal

portal vein flow was 25 cm/sec in sLTx versus 29 in wLTx. Blood flow calculated by vessel diameter and TAV showed no statistical difference between both groups.

Conclusion: Liver size after LTx is increased to the upper normal range, while blood flow velocities are below the normal range. The art. hepatica demonstrates changes regarding vascular resistance. sLTx leads to the same liver growth as wLTx and hemodynamic parameters exhibit no differences. In the view of long term functional adaption sLTx is not inferior to wLTx.

Zentrum Kinderheilkunde, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-30625 Hannover, Germany

31 5 α -REDUCTASE DEFICIENCY: CLINICAL, ENDOCRINE, AND MOLECULAR FINDINGS. O. Hiort, G. H. G. Sinnecker

Defects of the enzyme 5 α -reductase 2 are associated with a distinct and presumably rare form of male pseudohermaphroditism due to deficiency of dihydrotestosterone (DHT), which is necessary for the formation of male external genitalia. This enzyme with a length of 254 amino acids is encoded by the SRD5A2 gene, which is localized on chromosome 2 and divided into 5 exons. We have used leucocyte DNA from individuals with 46,XY karyotype and defective virilization to amplify the coding region of SRD5A2 with PCR. For the detection of point mutations, non-isotopic single strand conformation polymorphism analysis was performed and variations were directly sequenced. In 6 individuals from 5 families different homozygous point mutations with subsequent amino acid substitutions were detected. Phenotypes ranged from the characteristic female appearance with pseudovaginal perineoscrotal hypospadias in two individuals, predominantly male with severe hypospadias in three patients, to male with isolated micropenis in the brother of one male with hypospadias. Except for one female patient who had already undergone orchiectomy, the diagnosis was confirmed in each case by determination of pathologic testosterone (T) to DHT ratios after hCG stimulation. We conclude that point mutations in the SRD5A2 gene are associated with 5 α -reductase deficiency. The range of mutations is reflected by a broad spectrum of phenotypic variations in affected individuals. The finding of 5 α -reductase deficiency in patients with a common disorder of hypospadias or micropenis suggests that this disease may in fact be much more widespread than previously thought. It seems mandatory to determine T/DHT ratios in all patients with male pseudohermaphroditism. Molecular genetic analysis of the SRD5A2 gene may be an alternative diagnostic procedure for assessment of 5 α -reductase deficiency.

Klinik für Pädiatrie, Medizinische Universität zu Lübeck, Kahlhorststr. 31–35, D-23538 Lübeck, Germany

32 TERMINAL ALDOSTERONE BIOSYNTHESIS DEFECTS: BIOCHEMICAL AND MOLECULAR DIAGNOSIS. M. Peter¹, S. Geley², Rita Bernhardt³, R. Kofler², W. G. Sippell¹

Corticosterone methyl oxidase deficiency (CMO) is an autosomal recessively inherited disorder causing congenital isolated hypoaldosteronism due to defects in aldosterone synthase (P450aldo), the enzyme that converts corticosterone (B) to 18-hydroxycorticosterone (18-OHB) and aldosterone (Aldo). The gene encoding for this enzyme is termed CYP11B2. There are two inborn errors of terminal aldosterone biosynthesis characterized by overproduction of B and deficient synthesis of Aldo. CMO I is characterized by decreased production of 18-OHB while CMO II is characterized by overproduction of 18-OHB and an elevated plasma ratio of 18-OHB to Aldo. Affected infants present with severe salt-loss, failure to thrive, and frequent vomiting. In the last ten years, we diagnosed 16 infants with CMO deficiencies by simultaneous multisteroid analysis in a small plasma sample (RIA after extraction and automated high performance gel chromatography). Basal Aldo levels were decreased (range, 0.055–0.11 nmol/l) whereas B was elevated (range, 19–154 nmol/l). Plasma 18-OHB, ranging from 0.063–0.44 nmol/l, was decreased or in the lower normal range in 7 patients, whereas the other 7 patients had elevated 18-OHB levels (range, 12.1–57.7 nmol/l). 18-OH-DOC (range, 0.81–7.8 nmol/l) and DOC (range, 0.7–9.53 nmol/l) were elevated in all patients. In 7 patients, we found an elevated ratio of 18-OHB/Aldo (range, 286–900) and a low ratio of B/18-OHB (range, 1.1–5.8), whereas 7 other patients had a low 18-OHB/Aldo ratio (range, 1.1–6.95) and a high B/18-OHB ratio (range, 41–1360). To clarify the molecular basis of CMO I deficiency, we cloned and sequenced the CYP11B2 gene of a male Caucasian patient suffering from CMO I. We identified a single point mutation leading to

substitution of the highly conserved arginine to proline (R384P). Differential hybridization of mutation-specific oligonucleotide probes to PCR amplified CYP11B2 fragments revealed that both parents were heterozygous carriers for R384P while the patient appeared homozygous. Introduction of this mutation into a CYP11B2 cDNA expression vector construct and subsequent expression in COS cells revealed that R384P leads to a significant loss of P450aldo activity. Thus the R384P mutation provides a full molecular explanation for the CMO I deficiency in this patient and suggests that arginine 384 plays a major role in P450aldo function.

Dept. of Paediatrics, University of Kiel¹, Germany, Dept. of Molecular Biology, University of Innsbruck², Austria, Max Delbrück Center for Molecular Medicine³, Germany

33 INSULIN RECEPTOR MUTATIONS IN A NOVEL SYNDROME OF INSULIN RESISTANCE. P. Vorwerk, H. Vestergaard, C. T. Christoffersen, O. Pedersen, P. De Meyts

We have studied the structure and function of the insulin receptor (IR) in two brothers with a rare syndrome of congenital muscle fibre type disproportion myopathy (CFTDM) associated with severe insulin resistance. The IR gene of the patients was analysed by direct sequencing of PCR amplified IR-cDNA from Epstein-Barr virus (EBV) transformed lymphoblastoid cell lines as well as amplified genomic DNA fragments. The insulin binding to the EBV transformed lymphocytes of the patients was normal, but the number of IR on the cell surface was markedly decreased. We found two different mutations in the two alleles of the IR cDNA of both patients. The allele inherited from the patient's mother was alternatively spliced in exon 17 due to a point mutation in the –1 donor splice site of exon 17, resulting in a mixture of two mRNAs from that allele. The abnormal splicing out of exon 17 (exon 17-variant) shifts the amino acid (aa) reading frame and creates a stop codon 36 aa after exon 16. This leads to an IR truncated in the cytoplasmic part of the beta subunit and missing the entire tyrosine kinase domain. In the exon 17+ variant, the point mutation is silent and results in a normally transcribed IR. The allele inherited from the patient's father shows a missense mutation at position 1174 and changes in the tyrosine kinase domain an Arg into a Gln. In vitro overexpression of both mutated receptors shows no stimulated or basal autophosphorylation in presence or absence of insulin. The presence of all three cDNA variants demonstrates that the Gln¹¹⁷⁴ receptor as well as the truncated receptor and the wild type receptor are present in the lymphocytes of the patients. A third brother who inherited both normal alleles has a normal muscle phenotype and insulin sensitivity, suggesting a direct linkage of these IR mutations to the CFTDM phenotype.

Otto-von-Guericke-Universität, Medizinische Fakultät, Zentrum für Kinderheilkunde, Halberstädter Straße 13, D-39112 Magdeburg

34 SERUM PARAMETERS OF MATRIX METABOLISM AND OXIDATIVE STRESS IN ADOLESCENTS WITH TYPE I DIABETES. Th. Danne¹, C. Kittler¹, M. Herr¹, U. Rösigk², I. Schimke³, D. Schuppan⁴, B. Weber¹

Diabetic microangiopathy is associated with changes in extracellular matrix composition (decrease of laminin and vitronectin, increase of type VI collagen) and increased oxidative stress. We investigated these processes through serum parameters.

A matched pairs study was conducted. Samples of 60 adolescents with diabetes (age: 18 $\pm$ 3, diabetes duration: 10 $\pm$ 3 years; mean $\pm$ 1 SD) were drawn at onset of background retinopathy (by fluorescein angiography, n = 15) or microalbuminuria (≥ 2 of at least 3 measurements of overnight urine samples above 15 $\mu\text{g}/\text{min} \times 1.73 \text{ m}^2$, n = 15) and age, sex, and diabetes duration matched controls without complications (n = 15 each). They were assayed for Laminin (RIA, Behring), type VI collagen (ELISA), vitronectin (ELISA), malondialdehyde (MDA, endproduct of lipid peroxidation, thiobarbituric acid method), selenium (atom-absorption-spectrometry) and metabolic control (HbA1c, HPLC). Evidence for disturbed matrix metabolism was found, as 30% of the patients had elevated laminin levels (above 97. centile of healthy children) which were significantly related to metabolic control (r = 0.57, P = 0.000). However, no differences in any of the matrix parameters were found between patients developing complications and those without. Elevated MDA levels were found in 87% of the patients. Matched pairs comparison indicated significantly higher MDA levels in patients developing microalbuminuria (28 $\pm$ 7 vs. 18 $\pm$ 10 nmol/ml, P = 0.009). No associations of MDA levels with metabolic control, matrix parameters or selenium (an

essential component of antioxidant enzymes) were found. Interestingly, higher selenium levels were associated with poor metabolic control ($r = 0.49$, $P = 0.000$).

Laminin appears to be related to short-term derangements of matrix metabolism during poor glycemic control but does not give information on developing complications. On the other hand, since lipid peroxidation is one of the most important organic expressions of oxidative stress by the reactivity of oxygen free radicals, MDA levels in patients with diabetes may serve as an additional parameter to estimate an individual's risk for complications.

¹Kinderklinik (KAVH), FU Berlin, ²Hahn-Meitner-Institut Berlin, ³Bereich Medizin (Charité) der Humboldt-Univ., ⁴Klinikum Benjamin Franklin, FU Berlin, Heubnerweg 6, D-14059 Berlin

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ALTERED EXTRACELLULAR NEUROTRANSMITTER MILIEU IN HYPOTHALAMIC MEDIAL PREOPTIC AREA (MPOA) OF 5/6-NEPHRECTOMIZED RATS – IN VIVO EVIDENCE FOR CENTRAL DYSREGULATION OF GONADOTROPIC AXIS IN UREMIA. F. Schaefer, J. Kovács, A. Bandt, K. Schärer

A central nervous defect is presently discussed as the primary abnormality underlying disturbed pubertal development and fertility in chronic renal failure. We have recently identified a reduction of hypothalamic gonadotropin-releasing hormone (GnRH) secretion in the castrate uremic rat model. The pulsatile secretion of GnRH from neurons in the MPOA is regulated by a complex neuronal network. Catecholaminergic and serotonergic neurons exert predominantly stimulatory effects on GnRH release. To elucidate possible alterations of the local monoamine neurotransmitter milieu, we implanted stereotactically microdialysis probes in the MPOA of 5/6 nephrectomized and control rats. The animals had been orchidectomized to exclude confounding feedback effects by sex steroids. On the study day, the microdialysis probes were perfused continuously with artificial cerebrospinal fluid. After an equilibration period of 2 h, dialysate samples were collected every 10 min for 4 h in the awake, unrestrained animals. In each dialysate sample, norepinephrine (N), epinephrine (E), DHPG (the major catecholamine metabolite), DOPAC (dopamine metabolite) and 5-HIAA (serotonin metabolite) were measured by MPLC and electrochemical detection. The integrated mean dialysate concentrations of NE (0.22 ± 0.22 vs. 0.56 ± 0.41 pg/40 μ l tube) and E (0.65 ± 0.41 vs. 1.13 ± 0.53 pg/tube) were significantly reduced in the uremic animals ($P < 0.01$), whereas DHPG was increased (22.5 ± 7.5 vs. 12.8 ± 5.8 pg/tube). 5-HIAA concentrations were reduced in the uremic animals to 20% of the

concentrations observed in controls (1.3 ± 1.1 vs. 6.4 ± 4.5 pg/tube; $P < 0.0001$). DOPAC concentrations did not differ between uremic and control rats. Our results suggest deficient local release and altered metabolism of catecholamines, and the decreased 5-HIAA concentrations indicate low extracellular serotonin concentrations in the MPOA of uremic rats. In summary, our findings provide *in vivo* evidence for a deficient stimulatory input of superior neuronal systems to the hypothalamic GnRH pulse generator in experimental uremia.

Universitäts-Kinderklinik, Im Neuenheimer Feld 150, D-69120 Heidelberg

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LATE SYSTEMIC HYPERTENSION AFTER COARCTECTOMY IN CHILDHOOD: RELATIONSHIP TO HORMONES AND ANATOMY. J. Günthard, U. Zumsteg, D. Nufer, F. Wyler, U. B. Schaad

The long term complications of coarctation repair mainly consist in hypertension of the upper extremities either at rest or after exercise. Residual narrowing, as well as hormonal disorders, have been described as pathogenetic factors. 36 patients aged 13–35 yrs (10–28 yrs post coarctectomy) and 36 volunteers (16–34 yrs of age) underwent graded bicycle exercise testing. Systolic blood pressure (SBP) of the upper extremities was measured at rest and at peak exercise. Renin, adrenaline and noradrenaline were determined under standardized conditions, both at rest and after exercise. In addition, MR-imaging of the thoracic aorta was performed in the patients and revealed residual narrowing of $>30\%$ in 16 of them. Resting SBP in patients was 131 ± 14 vs 124 ± 9 mmHg in controls ($P = 0.01$), whereas peak exercise SBP was 239 ± 41 vs 209 ± 94 mmHg ($P < 0.0001$). Exercise-induced increase of plasma adrenaline was 30.6 pg/ml in patients vs 20.6 pg/ml in controls, statistically not different because of large inter-individual variation. However, the increase of plasma adrenaline correlated to the peak exercise SBP in the patient group ($r = 0.5$, $P = 0.037$). In the whole patient group, there was no correlation between either hormonal concentrations or blood pressure and residual narrowing, but the 16 patients with $>30\%$ residual narrowing showed a positive correlation between exercise-induced increase of plasma adrenaline and % of narrowing ($r = 0.74$, $P = 0.03$). Thus, besides anatomical narrowing, exercise-induced rise of plasma adrenaline may be implicated in the pathogenesis of late systemic exercise hypertension after coarctectomy in childhood. These results are in accordance with findings of increased adrenergic tone in essential hypertension and further support the adrenaline-hypertension hypothesis in man.

University Children's Hospital, CH-4005 Basel/Switzerland