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Arnerić M, Traven A, Starešinčić L, Sopta M. The retinoblastoma family of proteins directly represses transcription in Saccharomyces cerevisiae. J Biol Chem 2002;277: 8797-801.

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The retinoblastoma family of proteins are key cell cycle regulatory molecules important for the differentiation of various mammalian cell types. The retinoblastoma protein regulates transcription of a variety of genes either by blocking the activation domain of various activators or by active repression via recruitment to appropriate promoters. In this work the authors show that the retinoblastoma family of proteins functions as direct transcriptional repressors in a heterologous yeast system when fused to the DNA binding domain of Gal4. Mapping experiments indicate that either the A or the B domain of the pocket region is sufficient for repression in vivo. As is the case in mammalian cells, a phosphorylation site mutant of the retinoblastoma protein is a stronger transcriptional repressor than the wild type protein. The authors show that transcriptional repression by pRb is dependent on CLN3 in vivo. Furthermore, the yeast histone deacetylase components, RPD3 and SIN3, are required for transcriptional repression.

***Jelić M, Pećina M, Hašpl M, Kos J, Taylor K, Matičić D, et al. Regeneration of articular cartilage chondral defects by osteogenic protein-1 (bone morphogenetic protein-7) in sheep. Growth Factors 2001;19:101-13.**

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The efficacy of osteogenic protein-1 (OP-1; BMP-7) in regeneration of articular cartilage was examined by creating knee chondral defects in sheep. The recombinant BMP was delivered via an extra-articulary positioned mini-osmotic pump, which was fixed to the femoral diaphysis above the knee joint, and connected by a polyethylene tubing to the articular space. However, the biological activity of OP-1 released from pumps over a period of 2 weeks at 37° C was equal to ROS cell assay OP-1 standard. Following surgery, a total of 55 micrograms (low dose) or 170 micrograms (high dose) OP-1 in acetate buffer (pH 4.5) was slowly released from the pump over a period of 2 weeks. Twelve animals were operated, six of which were treated with the low OP-1 dose, and six with the high OP-1 dose. Three sheep of each group were killed either at 3 or 6 months following surgery, based on arthroscopical evaluation. The chondral defects in the control knees remained empty during the observation period. At 3 months following surgery, defects treated with both OP-1 doses were filled with connective tissue and cartilage. At 6 months following surgery, both doses of OP-1 stimulated regeneration in treated knees. The boundaries between new and old cartilage were well fused and mechanically resisted animals' weight bearing. The regenerated cartilage was rich in proteoglycans and type II collagen, as demonstrated by toluidine blue staining and immunohistochemistry. No signs of endochondral bone formation above the bony tidemark were observed. The authors suggest that a recombinant bone morphogenctic protein stimulates ingrowth of mesenchymal cells into the chondral defects which then transform into newly formed articular cartilage-like tissue.

***Sabolić I, Herak-Kramberger CM, Ljubojević M, Biemesderfer D, Brown D. NHE3 and NHERF are targeted to the basolateral membrane in proximal tubules of colchicine-treated rats. Kidney Int 2002;61:1351-64.**

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Depolymerization of microtubules in proximal tubule (PT) cells of colchicine-treated rats causes disruption of vesicle recycling and redistribution of some brush-border membrane (BBM) transporters into cytoplasmic vesicles. NHE3, an isoform of the Na^+/H^+ exchanger in the PT cell BBM, is acutely regulated by a variety of mechanisms, including protein trafficking and interaction with the PDZ protein, NHERF. The effects of microtubule disruption by colchicine on NHE3 trafficking in PT and the potential role of NHERF in this process have not been studied. Immunofluorescence and immunogold cytochemistry were performed on kidney tissue, and immunoblotting of BBM isolated from the renal cortex and outer stripe of control and colchicine-treated rats. In cells of the convoluted PT (S1/S2 segments) of control rats, NHE3 was located mainly in the BBM; subapical endosomes were weakly stained. In cells of the straight PT (S3 segment), NHE3 was present in the BBM and in lysosomes. In colchicine-treated rats, there was a marked redistribution of NHE3 from the BBM into intracellular vesicles and the basolateral plasma membrane in the S1/S2 segments. In the S3 segment, the abundance of BBM NHE3 was not visibly changed, but NHE3-positive intracellular organelles largely disappeared, and the antigen was detectable in the basolateral plasma membrane. The PDZ protein NHERF followed a similar pattern: in control animals, it was strong in the BBM and negative in the basolateral membrane in cells along the PT. After colchicine treatment, expression of NHERF in the basolateral membrane strongly increased in all PT segments, where it colocalized with NHE3. The data indicate that: (a) microtubules are involved in the apical targeting of NHE3 and NHERF in renal PT cells, and (b) the parallel basolateral insertion of NHE3 and NHERF may represent an indirect targeting pathway that involves transient, microtubule-independent basolateral insertion of these proteins, followed by microtubule-dependent, vesicle-mediated transcytosis to the BBM.

***Ćulić S, Jakobson A, Ćulić V, Kuzmić I, Šćukanec-Špoljar M, Primorac D. Etoposide as the basic and interferon-alpha as the maintenance therapy for Langerhans cell histiocytosis: a RCT. Pediatr Hematol Oncol 2001;18:291-4.**

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The treatment of patients who suffer from a disseminated form of Langerhans cell histiocytosis (LCH) is still controversial. So far, few larger randomized studies have been performed. The authors present 3 patients with a disseminated form of LCH - 4 months, 9 months, and 2 years old, respectively. The lesional Langerhans cells in each patient showed positive immunohistochemical reaction to S-100 protein and the presence of Birbeck granules was confirmed by electron microscopy. All the patients were treated with etoposide (VP-16), 200 mg/m^2 for 3 consecutive days, with 15 cycles at intervals of 3 weeks between each cycle, followed by maintenance therapy with IFN-alpha. All 3 patients reached complete stabile remission.

The patients were young, at high risk, with multiple-organ involvement of LCH, and two of them had obvious signs of organ dysfunction at presentation, suggesting a poor prognosis. All remain disease-free several years after therapy. The results suggest that INF-alpha may prevent recurrences in high-risk patients.

***Stipoljev F, Latin V, **Kos M, Mišković B, Kurjak A. Correlation of confined placental mosaicism with fetal intrauterine growth retardation. A case control study of placentas at delivery. Fetal Diagn Ther 2001;16:4-9.**

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The purpose of the study was to determine if the frequency of confined placental mosaicism in newborns with unexplained intrauterine growth retardation (IUGR) was higher compared with infants with appropriate growth in utero and the outcome of these pregnancies. A total of 20 cases with unexplained IUGR and 20 cases with appropriate growth for gestational age has been studied. Amnion, chorion and villi biopsy specimens were obtained from growth-retarded cases and controls at delivery. Cord blood specimens for 48-hour lymphocyte cultures were obtained from all infants with IUGR. Karyotype analysis revealed confined placental mosaicism in two of 20 (10%) cases with IUGR. In one growth retarded case and one appropriate growth for gestational age case, mosaicism was also confirmed in the amnion. Cytogenetic analysis from peripheral blood of newborns showed normal karyotype in all cases. Three pregnancies in the group of fetuses with IUGR (15%) ended with fetal death compared with normal fetal surveillance of all cases from the control group. Confined placental mosaicism was detected two times more frequently from placentas of growth- retarded infants compared with those of newborns with appropriate growth. The fetal loss was significantly higher in the group of cases with IUGR compared with the control group.

Rožmanić V, Velepić M, Ahel V, Bonifačić D, Velepić M. Prolonged esophageal pH monitoring in the evaluation of gastroesophageal reflux in children with chronic tubotympanal disorders. J Pediatr Gastroenterol Nutr 2002;34:278-80.

Pediatric Clinic and Clinic of Otorhinolaryngology, Rijeka University School of Medicine, Rijeka, Croatia

This study was designed to establish the frequency and ratio of pathologic gastroesophageal reflux (GER) in the development of chronic tubotympanal disorders. Twenty-seven children with chronic tubotympanal disorders (mean age, 2 to13 years; average age, 6.8 years) underwent 24-hour continuous pH monitoring. Fourteen of the examined patients had secretory otitis, whereas 13 patients had recurrent otitis. From each 24-hour pH monitoring, 12-hour daytime and nighttime periods were selected. Eleven patients were tested using simultaneous dual pH monitoring (distal and proximal pH monitoring). Fifteen (55.6%) of 27 patients had pathologic GER. The authors did not find a relation between pathologic GER and different types of ear disorders. Daytime pH monitoring yielded significantly more episodes of reflux than did nighttime monitoring. The reflux index was substantially higher during the day. In the current study, distal pH monitoring showed that 6 of 11 patients had pathologic GER, whereas proximal pH monitoring showed that only only 3 of 11 patients had pathologic GER. The authors recommend that a pH study be performed in children with chronic tubotympanal disorders when standard treatment is ineffective. The method of choice for the diagnosis of pathologic GER in patients with chronic tubotympanal disorders should be pH monitoring of the esophagus and throat.

Šubarić M, Mladina R. Nasal septum deformities in children and adolescents: a cross sectional study of children from Zagreb, Croatia. Int J Pediatr Otorhinolaryngol 2002;63:41-8.

Department of Otorhinolaryngology and Cervicofacial Surgery, Dubrava University Hospital, Zagreb, Croatia

The purpose of the study was to evaluate clinical data on the total prevalence of nasal septum deformities and particular types of deformity in children and adolescents. The study population consisted of 1797 randomly selected subjects divided into pre-school (aged 2-6), primary school (aged 7-14), secondary school (aged 15-18) and university (aged 19-22) groups. The observed pathologic septal deformities were classified into seven types according to Mladina's classification. The prevalence of nasal septum deformities according to age groups was 28.0% in the 2-6 group, 21.1% in the 7-14 group, 40.6% in the 15-18 group and 41.8% in the 19-22 group. The distribution of the seven types of septal deformity was 51.1%, 23.5%, 5%, 0.8%, 10.0%, 9.0% and 0.6%, respectively. The mean values (%) and 95% confidence intervals for the seven types of septal deformity were 14.7 (13.1-16.4%), 6.8 (5.6-7.9%), 1.4 (0.9-1.9%), 0.2 (0.0-0.4%), 2.9 (2.1-3.7%), 2.6 (1.9-3.4%) and 0.2 (0.0-0.4%), respectively. Total distribution in gender showed no difference ($p=0.102$). The authors conclude that in the youngest age group (2-6 years), types 1 and 2 deformities were exclusively found, whereas types 5 and 6 were found in older age groups. Since septal deformities can affect the growth and development of the maxilla and vice versa, the authors recommend examination of the nasal septum by an rhinologist who will be a part of a team performing the regular systematic health examination of children.

Zmajević M, Klarica M, Varda R, Kudelić N, Bulat M. Elimination of phenolsulfonphthalein from the cerebrospinal fluid via capillaries in central nervous system in cats by active transport. Neurosci Lett 2002;321:123-5.

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It was recently proposed that organic anions, such as cerebral acidic metabolites and phenolsulfonphthalein (PSP), are eliminated from cerebrospinal fluid (CSF) by diffusion into the central nervous system (CNS) and further by active transport into capillaries. To test this hypothesis, PSP was injected into cisternal CSF and its distribution into various parts of the CNS was measured 1 and 3 h later in control cats and those pretreated with probenecid, which blocks active transport of organic anions into capillaries. PSP in tissue shows an intensive pink color when exposed to 1 N NaOH. Planimetric analysis of color pictures of coronal CNS slices showed that at the first hour, diffusion and distribution of PSP into the CNS in both groups of animals was similar, while at the third hour, a great reduction of PSP distribution in the CNS in control and only a slight reduction in probenecid pretreated cats was observed. The results support the hypothesis that active transport across the capillary wall in the CNS is the main avenue for elimination of cerebral acidic metabolites from both CSF and CNS and in such a way that central homeostasis is maintained.

***Markotić A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, et al. Characteristics of puumala and Dobrava infections in Croatia. J Med Virol 2002;66:542-51.**

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In this study, two different hantaviruses, Puumala virus (PUUV) and Dobrava virus (DOBV), were demonstrated for the first time to coexist and cause hemorrhagic fever with renal syndrome (HFRS) in Croatia. Phylogenetic analysis showed some differences among the nucleotide sequences of PUUV originating from Dinara mountain, which was more closely related to Austrian PUUV than other Croatian PUUV from Mala Kapela mountain. More consistency was found among the Croatian DOBV. HFRS was verified in 85 of 201 suspected cases recorded in 1995 during the largest HFRS outbreak in Croatia. Most of these cases were soldiers. With the exception of the coastal region and islands, all of Croatia was found to be an area endemic for HFRS. A statistically significantly higher pro-

portion of DOBV-infected patients had acute renal failure, visual disturbance, severe thrombocytopenia, and elevated levels of nonsegmented leukocytes, creatine, and total bilirubin. The prevalence of gastrointestinal and electrocardiography disorders also was greater in DOBV-infected patients. Interestingly, significantly more PUUV-infected patients had elevated systolic blood pressure on admission to the hospital. Further prospective studies are necessary to shed more light on differences in HFRS severity associated with PUU and DOB viruses.

Tambić-Andrašević A, Tambić T, Kalenić S, Janković V. Working Group of the Croatian Committee for Antibiotic Resistance Surveillance. Surveillance for antimicrobial resistance in Croatia. Emerg Infect Dis 2002;8:14-8.

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The authors describe the activities of the Croatian Committee for Antibiotic Resistance Surveillance and report surveillance results for 1999. Twenty-two Croatian microbiology laboratories participated in the study. Resistance rates for the organisms isolated in different centers varied widely, but certain trends were apparent. Penicillin resistance in pneumococci (38%), methicillin resistance in Staphylococcus aureus (22%), the production of extended spectrum beta-lactamases by Klebsiella pneumoniae (21%), and imipenem resistance in Pseudomonas aeruginosa (11%) represent major resistance problems, especially in large hospitals. A comprehensive system of antimicrobial resistance surveillance, combined with training and external quality control programs, has identified high rates of resistance in key pathogens in some regions of Croatia. The program has heightened awareness of the problems of antimicrobial resistance and contributed to ongoing improvements in laboratory practice.

Iveković R, Mandić Z, Šarić D, Sonicki Z. Comparative study of pterygium surgery. Ophthalmologica 2001;215:394-7.

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The aim of this study was the comparison of recurrence rates of pterygia following different surgical methods. Twenty-one patients were operated by the bare sclera technique, with the application of mitomycin C (0.25 mg/mL for 2 min). Twenty-seven patients were operated by limbal stem cell transplantation. The third group contained 13 patients who were operated by amniotic membrane transplantation. Five recurrences of pterygia were noted in the mitomycin C group and 2 each in the limbal stem cell transplantation group and the amniotic membrane group. The mean time to recurrence was 5.4 4.12 months in the mitomycin C group versus 10.5 3.5 months in the group of patients with limbal transplantation. In the third group, both recurrences were evident 4 months after the operation.

***Marinko D, Dragutin K, Bašić-Kes V, Šerić V, Demarin V. Transcranial Doppler sonography for post-traumatic stress disorder. Mil Med 2001;166:955-8.**

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Transcranial Doppler sonography (TCD) was used to examine the mean speed of blood circulation in 50 patients suffering from post-traumatic stress disorder (PTSD). The sonography was repeated 6 months after successful psychiatric treatment. Doppler sonography of Willis's circle blood vessels and vertebrobasilar flow was performed on healthy controls as well. All of the subjects in both groups were 20 to 43 years old and had not suffered from other diseases. Vasospasm of Willis's circle blood vessels was discovered in 62% of PTSD patients, which decreased to 22% after treatment. In the control group, it occurred in 8% of subjects. TCD examination of vertebrobasilar system blood vessels did not identify significant differences in blood circulation mean speed between controls and PTSD patients, regardless of whether they had or had not been treated. This research proved the value of TCD in discovering Willis's circle blood vessel vasospasm in PTSD patients, which suggests the inclusion of TCD in diagnosing PTSD. The value of TCD was affirmed in controlling treatment success, because after 6 months of psychiatric treatment, there were significantly fewer patients with Willis's circle blood vessel vasospasm.

Alajbeg I, Krnjević-Pezić G, Smeh-Škrbin A, Vržogić P, Vučićević-Boras V, Dobrić I, et al. Non-aromatic naphthalane preparation: preliminary clinical study in the treatment of psoriasis vulgaris. J Pharm Biomed Anal 2001;26:801-9.

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This study aimed to prove the similarity of the composition of non-aromatic Croatian naphthalane (NAN) with brown naphthalane (BN), which is used in the treatment of psoriasis vulgaris. The comparison of the compositions was performed by obtaining GC fingerprints, which were supported by GC-MS data. In spite of remarkable differences in general profiles of the GC chromatograms, lower and medium molecular weight components of NAN were found to be qualitatively the same as the saturated constituents of BN. Quantitatively, lower molecular weight components as well as all n-alkanes were comparatively lower in NAN. NAN, additionally, contained higher molecular weight components, among which there were saturated oligocyclic hydrocarbons (up to pentakishomohopanes), described as responsible for the curing effect of naphthalane. The composition characteristics of NAN including its non-aromatic character made it suitable for a clinical study. In the treatment, the efficacy was determined by means of comparison of Psoriasis Area Severity Indices, PASI, at the beginning and at the end of the therapy. Adult volunteer-patients, nine males and six females, applied NAN over the whole body, except the scalp, at the room temperature for 20 min and this was followed by the selective UVB radiation. After the 3-week therapy, all essential clinical manifestations as erythema, desquamation and infiltration were significantly reduced in 14 patients; in nine cases the improvement was 50-93%, while the state of five patients improved between 25 and 50%. In one case, there was no obvious change. No exacerbation occurred during the therapy period. No adverse effect on hematological or biochemical parameters was noticed.

Lukić IK, Glunčić V, Katavić V, Petanjek Z, Jalšovec D, Marušić A. Weekly quizzes in extended-matching format as a means of monitoring students' progress in gross anatomy. Ann Anat. 2001;183:575-9.

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We compared weekly quizzes in extended-matching format with multiple-choice questions and oral examinations as means of monitoring students' progress in gross anatomy. Students' performance on 19 weekly oral examinations or 10-question quizzes based on extended-matching or multiple-choice formats were correlated with their success on 3 interim examinations and the final comprehensive examination. The Kuder-Richardson formula 20, an estimate of precision of the test, was 0.64 for extended-matching quizzes. Students' performance on interim examinations did not differ significantly. There was a significant correlation between students' mean scores on weekly quizzes and mean scores on interim examinations in both the extended-matching ($r=0.516$) and multiple-choice group ($r=0.823$). The mean grades (ranging from 2 to 5) on the final exam, based on understanding of anatomical concepts and their application in clinical practice, were significantly higher in extended-matching group (4.8) than in the multiple-choice (4.1) and orally examined groups (3.9) ($p<0.05$). We conclude that extended-matching quizzes were at least as effective as multiple-choice quizzes and oral examinations and may be better for acquiring synthetic understanding of anatomical concepts especially in combination with other means of knowledge assessment. We recommend them as a reliable and objective means of monitoring students' performance during a gross anatomy course.

Trobonjača Z, Radošević-Stašić B, Crnčević Z, Rukavina D. Modulatory effects of octreotide on anti-CD3 and dexamethasone-induced apoptosis of murine thymocytes. Int Immunopharmacol 2001;1:1753-64.

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In an attempt to elucidate the effects of somatostatin on two crucial processes that regulated T-cell differentiation and selection in thymus in this study, the authors investigated in vivo and in vitro the effects of octreotide (SMS 201-995) on dynamics of apoptosis, induced by dexamethasone (DEX) or by anti-CD3 monoclonal antibodies (mAb). The data were estimated by analysis of absolute cellularity, DNA fragmentation and maturational stage of thymocytes, detecting the CD4 and/or CD8 and T cell receptor (TCR) expression on thymocytes. The results, obtained by estimation of subdiploid peak of DNA and ladder DNA formation, have shown that SMS given in vivo, may potentiate the early phase of DEX-induced nuclear fragmentation (at 24 h), accelerating simultaneously the elimination of thymic cells with double positive (DP) CD4highCD8 high phenotype (expressed both as percentage and absolute number). On the contrary, SMS, given both in vivo and in vitro, down-regulated the late process (at 72 h) of nuclear fragmentation, induced by anti-CD3 mAb, minimizing simultaneously the elimination of DP cells (expressed both as percentage and absolute number). In anti-CD3-treated cultures of thymocytes, SMS retarded also the elimination of immature thymocytes, expressing the TRC alpha/betalow or intermediate phenotype. The data emphasize that octreotide might have important regulatory effect on processes of thymic differentiation and maturation, which are crucial for T cell selection, induction of tolerance and prevention of autoimmune diseases.