

Baršić B, Maretić T, Majeruš L, Strugar J. Comparison of azithromycin and doxycycline in the treatment of erythema migrans. *Infection* 2000;28:153-6.

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A randomized, multicenter, open clinical trial was undertaken in order to compare the efficacies of azithromycin and doxycycline in the treatment of patients with *Lyme disease* associated with erythema migrans. A total of 48 patients was treated orally with azithromycin, 500 mg bid on the 1st day, followed by 500 mg once daily for the next 4 days or doxycycline (40 patients) 100 mg bid for 14 days. Intention-to-treat analysis of clinical efficacy showed no difference between the two treatment regimens. Clinical success was observed in 46 (95.8%) azithromycin- and 33 (82.5%) doxycycline-treated patients, ($p=0.0737$). Minor symptoms persisted or appeared in the posttreatment period in two of 47 azithromycin- and three of 35 doxycycline-treated patients ($p=0.646$). Major manifestations appeared only in two patients in the doxycycline group ($p=0.179$). There was no difference in the tolerability of both drugs. In conclusion, azithromycin (a total dose of 3 g) is equally effective as standard doxycycline treatment for erythema migrans in adult patients.

Bendelja K, Gagro A, Baće A, Lokar-Kolbas R, Krstulović-Hrešić V, Draženović V, et al. Predominant type-2 response in infants with respiratory syncytial virus (RSV) infection demonstrated by cytokine flow cytometry. *Clin Exp Immunol* 2000;121:332-8.

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It has been proposed that RSV infection stimulates type-2 cytokine responses, resembling those found in atopy and asthma. Peripheral blood cells were obtained from RSV-infected infants ($n=30$) and healthy controls ($n=10$). After *in vitro* restimulation of the cells, intracellular IL-4 and interferon-gamma (IFN-gamma) were measured by flow cytometry. The cells from RSV-infected infants produced more IL-4 and less IFN-gamma than those from healthy controls. IL-4 production was more frequent in CD8 than in CD4 cells, and the bias toward IL-4 production was greatest in infants with mild infections, whereas IFN-gamma production increased with disease severity. The conclusion is that RSV infection is associated with IL-4 production in peripheral T cells and that peripheral blood in infants with severe disease may be depleted of cytokine-producing cells.

Crabtree N, Lunt M, Holt G, Kroger H, Burger H, Grazio S, Khaw KT, Lorenc RS, Nijs J, Stepan J, Falch JA, Miazgowski T, Raptou P, Pols HAP, Dequeker J, Havelka S, Hozowski K, Jajic I, et al. Hip geometry, bone mineral distribution, and bone strength in European men and women: the EPOS Study. *Bone* 2000;27:151-9.

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The authors used Lunar DPX "beta" versions of hip strength analysis (HSA) and hip axis length (HAL) software to analyze scans from ten representative age-stratified population samples in the European Prospective Osteoporosis Study (EPOS). All 1617 subjects were >50 years of age, and 1033 were women. The bone mineral density of the upper half of the femoral neck declined at a faster rate with age than that in the lower half. Femoral neck cross-sectional moment of inertia (CSMI), a measure of resistance

to bending, showed no significant age reduction in either gender. However, height and weight effects on CSMI were significantly more beneficial in men than in women ($0.002 < p < 0.012$) and the weight effect appeared to be mediated by bone mineral content (BMC). Compressive stress (Cstress), defined as the stress in the femoral neck at its weakest cross section arising from a standardized fall, was higher in women. Although Cstress increased with body weight when BMC was held constant, in practice it fell through the association and statistical interaction of rising body weight with rising BMC. HAL, as expected, was strongly positively associated with male gender and also height ($p < 0.0001$). Hip strength-related indices were markedly center-dependent. Significant differences ($p < 0.0001$) were noted between the centers for all the variables investigated that related to hip geometry. Adjustment for femoral neck bone mineral content (totBMC) showed these center differences to account for >50% of center variation in hip strength, which remained highly significant ($p < 0.0001$). The authors conclude that there are substantial geographical differences in femoral neck geometry as well as in BMD. These geometric variations may contribute to the large variations in hip fracture risk across Europe.

Eraković V, Župan G, V arljen J, Laginja J, Simonić A. Lithium plus pilocarpine induced status epilepticus - biochemical changes. *Neurosci Res* 2000;36:157-66.

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The aim of the study was to investigate the changes in biochemical mechanisms facilitating cellular damages in the lithium plus pilocarpine treatment and the resulting status epilepticus. The whole brain free fatty acid (FFA) level as well as the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutamate dehydrogenase, aspartate-aminotransferase (AST), alanine-aminotransferase, gamma-glutamoyl transferase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase in the frontal cortex, cerebellum hippocampus and pons-medulla region of Hannover-Wistar rats were determined. The control group was intact with no previous experimental history. LiCl (125 mg/kg ip.) was injected 20 h prior to pilocarpine (30 mg/kg ip.) and the treated rats were sacrificed 1 or 2 1/2 h after pilocarpine administration. The results show that lithium plus pilocarpine administration and the resulting status epilepticus produced the significant increase of the brain FFA content. Decreased GPX activities were detected in the frontal cortex, cerebellum and hippocampus of the treated rats without the accompanying decrease of SOD activity. Increased AST and LDH activities were observed in the frontal cortex, increased soluble ALP activities in the frontal cortex and pons-medulla region whereas the increased activity of membrane bound ALP was detected in the hippocampus of the rats with status epilepticus. The data indicate clear regional differences of biochemical changes caused by lithium plus pilocarpine treatment and the resulting status epilepticus, frontal cortex bring the most affected site.

Grdiša M, White MK. Erythrocytic differentiation and glyceraldehyde-3-phosphate dehydrogenase expression are regulated by protein phosphorylation and cAMP in HD3 cells. *Int J Biochem Cell Biol* 2000;32:589-95.

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The authors have employed the temperature-sensitive chicken erythroblast cell line HD3 that differentiates to the erythrocyte phe-

notype at 42^o C in the presence of inducers (hemin and butyric acid). The role of tyrosine and serine/ threonine phosphorylation pathways were evaluated with the phosphatase inhibitors sodium vanadate and okadaic acid, respectively. In the presence of phosphatase inhibitors, HD3 cells underwent differentiation and increased their synthesis of hemoglobin which is a marker protein for red blood cells differentiation. The levels of both GAD mRNA and enzymatic activity were increased by phosphatase inhibitors. The role of cAMP in differentiation was also assessed. Differentiation of HD3 cells was associated with an increase in cAMP. However the phosphodiesterase inhibitor IBMX was not a good inducer of hemoglobin synthesis but did induce GAD mRNA and enzymatic activity. Together these results suggest that multiple pathways (including serine/threonine phosphorylation, tyrosine phosphorylation and elevated cAMP) are involved in the regulation of erythroblastic differentiation, hemoglobin synthesis, GAD gene expression and GAD activity in HD3 cells.

Jernej B, Banović M, Čičin-Šain L, Hranilović D, Balija M, Orešković D, et al. Physiological characteristics of platelet/circulatory serotonin: study on a large human population. *Psychiatry Res* 2000;94:153-62.

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The aim of this work was the study of platelet/circulatory serotonin (5-HT), specifically alternative ways of its measurement and main physiological characteristics. The study was performed on a large human population (n=500) of blood donors of both sexes over the course of a longer time period (17 months). Owing to the heterogeneity in measurement of circulatory serotonin in the literature, three ways of expression were comparatively studied: per unit number of platelets, per unit mass of platelet protein and per unit volume of whole blood. Results demonstrated unimodal distribution of individual frequencies of platelet/circulatory serotonin in the human population with the mean values of 579 ± 169 ng 5-HT/10(9) platelets; 332 ± 89.9 ng 5-HT/mg protein and 130 ± 42.3 ng 5-HT/ml blood (mean ± sd.). A progressive decrease of serotonin level with age (18-65 years) was demonstrated, reaching statistical significance between the extreme age groups. No significant differences in the serotonin level between the sexes were observed. No seasonal oscillations in platelet/circulatory serotonin were found. Finally, regarding the methodology of measurement, the results demonstrated a good correlation among the above-mentioned ways of expression of platelet/circulatory serotonin. This indicates the possibility of intercomparison of the literature reports expressing this physiological parameter either as 5-HT concentration in platelets or as 5-HT level in the circulation.

Muro AF, Marro ML, *Gajović S, Porro F, Luzzatto L, Baralle FE. Mild spherocytic hereditary elliptocytosis and altered levels of alpha- and gamma-adducins in beta-adducin-deficient mice. *Blood* 2000;95:3978-85.

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Adducin is one of the proteins localized at the spectrin-actin junction of the membrane skeleton. In this work the authors show that deficiency of beta-adducin produces an 80% decrease of alpha-adducin and a fourfold up-regulation of gamma-adducin in erythrocytes. Beta-adducin or any other isoform generated by translation of abnormally spliced messenger RNAs could not be detected by antibodies either in ghosts or in cytoplasm of -/- erythrocytes. Actin levels were diminished in mutant mice, suggesting alterations in the actin-spectrin junctional complexes due to the absence of adducin. Elliptocytes, ovalocytes, and occasionally spherocytes were found in the blood film of -/- mice. Hematological values showed an increase in reticulocyte counts and mean corpuscular hemoglobin concentration, decreased mean corpuscular volume and hematocrit, and normal erythrocyte counts that, associated to splenomegaly, indicate that the mice suffer from mild anemia with compensated hemolysis. These modifications are due to a loss of membrane surface and dehydration that result in an increase in the osmotic fragility of red blood cells. The marked alteration in osmotic fragility together with the predominant presence of elliptocytes is reminiscent of the human disorder called spherocytic hereditary elliptocytosis. The results suggest that the amount of adducin remaining in the mutant animals

(presumably alpha gamma adducin) could be functional and might account for the mild phenotype.

Paladino J, Štimac D, Rotim K, Pirker N, Štimac A. Ultrasonic contact microprobe: experimental results. *Minim Invasive Neurosurg* 2000;43:72-4.

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At the Department of Neurosurgery, School of Medicine, University of Zagreb, the original ultrasonic contact microprobe (UCM) was designed. The efficacy of the instrument was tested on 120 brains of Wistar strain rats. The authors have been investigating the possibility of transferring high-energy ultrasound through the titanium wire probe of the device and the efficacy of UCM in controlled punctiform destruction of brain tissue. Light and electron microscope assessed the lesions made in the brains of experimental rats. Histological findings in the preparations showed the zone of thermal injuries from 100 to 200 μm and the zone of ultrastructural changes from 200 to 300 μm, indicating the sparing effect of the microprobe with regard to the adjacent neurovascular structures. The small dimensions of the ultrasonic contact microprobe (1.6 mm) enable its introduction through the operating canal of a ventriculoscope. Further research is expected to show the efficacy of the ultrasonic contact microprobe in endoscopic neurosurgery.

Prebeg Ž, Bralić I. Changes in menarcheal age in girls exposed to war conditions. *Am J Hum Biol* 2000;12:503-8.

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The purpose of this study was to assess changes in mean menarcheal age of girls in the city of Šibenik in the period from mid-1980s to the mid-1990s. Šibenik is a Dalmatian town which was exposed to hard war conditions in 1991-1995. Menarcheal status of Šibenik girls was surveyed three times, in 1981, 1985, and 1996, and included 720, 1207, and 1680 girls, respectively, ages 9.5-16.5 years. Results show a slight decrease in menarcheal age from 1981 to 1985 (from 12.97 ± 0.06 years to 12.87 ± 0.05), and then a significant increase from 12.87 ± 0.05 years in 1985 to 13.13

0.10 years in 1996. The increase in mean menarcheal age occurred in all socioeconomic groups based on parental occupation and number of siblings. In the group of girls whose homes were damaged during war, menarche occurred at an average of 13.53 ± 0.14 years, while those who lost a family member experienced menarche at an older mean age, 13.76 ± 0.27 years. However, when the girls who experienced personal tragedies were excluded the onset of menarche was still later than in girls surveyed in the earlier periods. The results suggest that the reversal in the secular trend of menarcheal age in Šibenik girls can be attributed to psychological pressures and uncertainties associated with conditions of war.

Roknić S, Glavaš-Obrovac L, Karner I, Plantanida I, Zinić M, Pavelić K. In vitro cytotoxicity of three 4,9-diazapyrenium hydrogensulfate derivatives on different human tumor cell lines. *Chemotherapy* 2000;46:143-9.

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The authors synthesized three potential novel intercalators - 4,9-diazapyrenium hydrogensulfate derivatives: 5,10-diphenyl-4,9-dimethyl-4,9-diazapyrenium hydrogensulfate (FDAP), 4,9-dimethyl-4,9-diazapyrenium hydrogensulfate (GDAP) and 2,4,7,9-tetramethyl-4,9-diazapyrenium hydrogensulfate (MDAP) and tested their biological effects *in vitro* on four human tumor cell lines (SKBr3: breast carcinoma, HeLa: cervical carcinoma, CaCo2: colon carcinoma and SW620: poorly differentiated cells from lymph node metastasis of colon carcinoma). Cytotoxic effects on cell growth and viability were determined using tetrazolium dye (MTT) assay. DNA synthesis and proliferation of treated cells were studied by the [³H]-thymidine incorporation test. DNA fragmentation was analyzed by agarose gel electrophoresis. The growth inhibitory effect was cell-specific and dose-dependent. The most pronounced antiproliferative effect was observed on SKBr3 cells for FDAP (10⁻⁵ M) 91.8%, for MDAP (10⁻⁵ M) 85.3% and on

SW620 cells for GDAP (10^{-5} M) 65.3%. The DNA ladder fragmentation of treated HeLa and SKBr3 cells, as a hallmark of apoptosis, was observed. Based on specific DNA fragmentation, morphological changes and growth inhibition of treated human tumor cells it was concluded that tested substances induced apoptotic cell death.

Rubelj I, Huzak M, Brdar B. Sudden senescence syndrome plays a major role in cell culture proliferation. Mech Ageing Dev 2000;112:233-41.

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Normal human cells of various types have a finite and predictable proliferative potential in vitro. This limited life span is due to a gradually increasing fraction of senescent cells that appear in the culture in a sudden and stochastic fashion due to a phenomenon referred to as sudden senescence syndrome (SSS). Because nondividing cells increasingly accumulate in the culture, dividing cells have to compensate for nondividers in order to accomplish additional population doubling (PD). Thus, individual dividing cells undergo more divisions, called cell generations (CG), than the number of PDs. Based on integrated experimental data, we calculated maximum CG for normal human diploid fibroblasts (HDF). It appears that for a HDF culture that undergoes 65 PD, the calculated final CG is at least 126. Based on the obtained value for CG we calculated the total size of the culture, both with and without effect of SSS. If no SSS takes place and cells divide by geometrical progression, the culture will grow up to 2^{126} or 10^{38} cells. By constantly eliminating cells from further divisions, causing cell loss (CL), SSS reduces the total size of the culture at every point during its proliferation. Accordingly, by preventing virtually every cell in the culture from reaching its original maximum doubling capacity, SSS appears to be the most important mechanism that influences cell culture proliferation.

Šimić G, Šešo-Šimić D, Lucassen PJ, Islam A, Krsnik Z, Cviko A, et al. Ultrastructural analysis and TUNEL demonstrate motor neuron apoptosis in Werdnig-Hoffmann disease. J Neuropathol Exp Neurol 2000;59:398-407.

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Werdnig-Hoffmann disease (WHD) is the most severe clinical type of spinal muscular atrophy characterized by loss of lower motor neurons and paralysis. The authors examined the hypothesis that disease pathogenesis is based on an inappropriate persistence of normally occurring motor neuron programmed cell death. The diagnosis of WHD was made on the basis of clinical findings, electromyoneurography, and biopsy, and further confirmed by mutation analysis of the survival motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) genes using PCR. They used ultrastructural analysis as well as TUNEL and ISEL methods to assess DNA fragmentation, and immunocytochemistry to identify expression of the apoptosis-related proteins bcl-2 and p53. A significant number of motor neurons in the spinal cord of children

with WHD were shown to die by apoptosis. As revealed by TUNEL, dying neurons in WHD patients comprised 0.2%-6.4% of the neuron numbers counted. This finding contradicts earlier studies that failed to find such evidence and suggests that early blockade of prolonged neuron apoptosis may be a potential therapeutic strategy for WHD.

Škrablin S, Kuvačić I, Pavičić D, Kalafatić D, Goluža T. Maternal neonatal outcome in quadruplet and quintuplet versus triplet gestations. Eur J Obstet Gynecol Reprod Biol 2000;88:147-52.

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The aim of the study was to compare the natural histories of triplet versus quadruplet and quintuplet gestations. A retrospective study included 64 multifetal pregnancies (52 sets of triplets, 9 sets of quadruplets and 3 sets of quintuplets) cared for during past 12 years. Quintuplets and quadruplets were compared with triplet pregnancies according to gestational age, birthweight, pregnancy complications, and perinatal outcome. Student's t-test, Fisher exact test and χ^2 test were used for statistical analysis. Although mean gestational age at delivery between triplets and higher order gestations was not significantly different, birthweight of quadruplets and quintuplets was significantly lower. Pregnancy complications, including intrauterine growth retardation, were equally distributed between the groups. Early neonatal and perinatal mortality were significantly higher in quadruplets and quintuplets than in triplets. Surprisingly, survival of growth retarded fetuses was better than survival of their eutrophic counterparts. The spontaneous loss rate was 11.5% for entire triplet gestation and 16.7% for quadruplet and quintuplet pregnancies. In conclusion, as the spontaneous loss rate of triplets and higher order pregnancies observed in this study is quite similar to pregnancy loss rate caused by multifetal pregnancy reduction, conservative management of multifetal pregnancies in specialized tertiary centers seems to be a prudent solution.

Uravić M, Milotić F, Depolo A, Rahelić V, Petrošić N. Colorectal war injuries. Milit Med 2000;165:186-8.

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During the war in Croatia (1991-1995), 851 war casualties were treated by the surgical teams of Rijeka Clinical Hospital. Among them, 66 patients (7.8%) had colon and/or rectum injuries, usually combined with trauma of other abdominal organs, most often jejunum and ileum (24.2%). Regarding the site of injury, right and sigmoid colon were the most frequently traumatized areas (30.3% and 31.8%, respectively). All patients received surgical treatment within 3 hours from wounding. In 89% of patients, relieving colostomy was formed after the resection of the damaged part of the intestine; 11% of patients were treated with primary resection or sutures without colostomy. The mortality rate of 3% (2 patients) was caused by mutilating multiple organ abdominal trauma combined with massive brain injury.