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The 16th Conference about Laboratory Animals organized by the Czech Laboratory Animal Science Association (SVLZ) was held at Kutna Hora in the beautiful historic city of the Central Bohemia, May 6 – 8, 2013. A total 61 of scientists participated in the meeting. Most papers were devoted to laboratory animal protection and welfare, several presentations reported new data from experiments both in animals and new alternative models. The amended laws and regulations in the care of laboratory animals were presented too.

USING OF HORMONAL CONTRACEPTION IN RHESUS MONKEYS FEMALES

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Makak rhesus (macaca mulatta) of the long-tail monkey family belongs among the most extended subhuman primates used in the area of research and experiments, and its breeding in accredited facilities is the most suitable alternative, as compared with catching the animals from wildlife, which is connected with health risks, the animals' stress of the catching, transport and subsequent use. Nevertheless, unplanned mating of the animals in such facility can be undesirable, be it for the reason of mating between closely related individuals, health status or other reasons. With regard to unwanted mating of 8 females of makak rhesus in the age of 4-5 years in the accredited breeding facility and their planned use in experiments, it was decided to adopt a one-off contraceptive preparation containing synthetic levonorgestrel (Postinor-2). Levonorgestrel is a representative of the group of 13-etylgonan derivatives; thanks to its ethyl group at 13-position, it has pronounced gestagenic effects; in plasm, 93-95 % of it is linked with proteins (first of all SHBG), and it is excreted from organism in the form of reduced metabolites, predominantly by conjugation with glucuronic acid or sulfuric acid. On the basis of the tests carried out on primates, available literature, as well as consultation with veterinary surgeon and with the supplier of the medicine chosen, it was decided to use a preparation containing 750 µg of synthetic levonorgestrel (Postinor-2). In human medicine, this preparation is administered in two steps, regardless of body weight. The first tablet is administered within 72 hours after sexual intercourse and the second within 12 hours after the first one. With regard to the animals' body weight, the chosen dose was ¼ of tablet containing 187.5 µg of levonorgestrel, administered orally in a banana. The first dose was accepted without problems by 7 animals out of 8; the remaining animal accepted it one day later. The second dose after 12 hours was accepted without problems. Diagnostics of potential pregnancy in makak rhesus is possible by palpation from the 16th day of gravidity, or by ultrasound or determination of hormonal changes in blood and urine. The subsequent check of efficiency was carried out by determination of choriongonadotropine (hCG) using the immune-enzymatic analysis from blood serum, which had negative results (<1 IU/l); this analysis was performed two weeks after the administration of the second dose. The detection limit of this method is 0.5 IU/l; the values above 5 IU/l indicate a positive test. The similarity and biological activity of human hCG molecule are close to those of mammals, large primates exhibiting a minimum number of differences. According to literature, the production of choriongonadotropine in makak rhesus can be detected 12 days after the conception, and it starts to decrease 20 days after the conception. The negative result was confirmed subsequently by ultrasound examination, 2 months after the administration of contraceptive. The administration of the preparation containing levonorgestrel did not cause any health problems (nausea, diarrhea, pain), and the animals underwent standard spring rutting season accompanied by typical outer symptoms, such as bulging of skin of the caudal area and legs, and flare in the area of outer sexual organs.

THE LEGAL STATUS OF LABORATORY ANIMALS IN THE CZECH REPUBLIC OR SO IT GOES

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Life is change and thus the area of experimental animals has significant changes. Civil Code will apply from 1 January 2014 by Act No. 89/2012 Coll. Live animals will no longer objects (§ 494) as opposed to the Act No. 40/2009 Coll., The Criminal Code (§ 134) and will remain so in the future. Amended by Act No. 246/1992 Coll., The protection of animals against cruelty (especially §§ 15-18), Decree No. 419/2012 Coll., On the protection of animals and the Decree No. 22/2013 Coll., On education the protection of animals against cruelty (especially § 10-15) has been in force since January 2013. Unfortunately, the laws are more stringent than required by the Directive of the European Parliament and of the Council 2010/63/EC on the protection of animals used for scientific purposes. It should be noted that our legal system is in full compliance with European international law. Multilateral European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes, including the Additional Protocol of the Council of Europe and the Directive are already accepted by our legal system.

THE IMPORTANCE OF ANIMAL RESEARCH IN THE HISTORY OF LANGERHANS ISLET TRANSPLANTATION. MILESTONE OF LANGERHANS ISLET LABORATORY

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The role of animal experimentation has been vital in the research and later practical application in therapeutic use. The history of Langerhans Islets transplantation began in 1869, with Paul Langerhans discovering islets in animal models but their function remained unknown. E.L. Opie, in 1901, also using animal models explained the islets function and in 1902 L.W. Ssobolew demonstrated that islets were responsible for sugar distribution. Later in 1904, Jean De Meyer named the products of islets as "insulin". The breakthrough in islet research came in 1965 with S. Moskalewski employing rats for the isolation and culture of islets. The next significant step was in 1967 by P.E. Lacy and M. Kostianowsky who isolated intact islets from the rat pancreas. In 1971, C. Ricordi and his team started research about the method of islet transplantation from various animals. The next year, P.E. Lacy surgically transplanted islets between animals. In 1988, Ricordi developed the pioneering method for mechanical isolation of large amounts of islets very easily firstly from pigs and later from humans. The most recent development in 2000 in Edmonton, Canada, was when a research team reported seven Type 1 diabetic patients released from daily insulin injections after islet transplantation. Initial phases of this research performed on animals allow for translation of ideas to clinical practice.

USING OF PROBIOTICUM PROBIOSTAN IN BROILER RABBITS NUTRION

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Digestive disorders account for 70 % of all rabbit diseases. These disorders cause high morbidity which brings about higher economic losses than rabbit death. The most using protection against rabbit indigestion is the antibiotic administration. However, antibiotics can produce side effects and leave residues in treated animals meat. That is why it is necessary to find out antibiotic alternatives. One of the possibility of antibiotic substitution is probiotic administration. For that reason the aim of this study was to evaluated the influence of probioticum PROBIOSTAN (fermented preparation supplemented with yeast and lactobacilli produced by Biokron s.r.o.) on broiler rabbit growth and health status. Two experiments with one hundred and fifty HYLA broiler rabbits weaned at 35 days of age were used in this study. Animals were fed by standard diet for rabbit fattening *ad libitum*. Rabbits were divided to two groups (I and II) in each of experiment. The diet of group I from both experiment 1 and experiment 2 was supplemented with probioticum PROBIOSTAN in recommended amount 2 g.kg^{-1}. Each experiment was started at 42 days of rabbits age and finished by the achievement of 2600 g of live weight (no later than at 84 days of rabbits age). Both rabbit growth performance and feed consumption were not statistically different in groups with PROBIOSTAN supplement and groups without this supplement. Health status was better in groups with PROBIOSTAN supplement. In these groups the significantly lower morbidity were recorded. Our results shows that the probioticum PROBIOSTAN supplement can be convenient substitute for chemical drugs commonly used in broiler rabbit fattening.

EUTHANASIA OF LABORATORY ANIMALS FROM THE VIEWPOINT OF THE ANESTHESIOLOGIST

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The euthanasia is an integral part of work with experimental animals. At present, there are many methods of euthanasia, which are divided into physical and pharmacological methods. This course gives an overview of the various methods of euthanasia, their advantages and disadvantages. The particular attention is paid to the use of carbon dioxide and the new knowledge about the mechanisms of action. There is presented also the effects of other gases, nitrogen and argon. The rodents have sensors that are highly sensitive to reduced oxygen concentration. Even the short term inhalation anesthetics such as isoflurane and sevoflurane after euthanasia leave residues in the body. Therefore thus the sacrificing animals for example pig, is inappropriate for human consumption. The use of narcotic drugs for euthanasia altered their pharmacokinetics during the dying. This problem is discussed in detail in connection with executions injections in USA. The difficulties with drug administration for these special circumstances are discussed in the lecture.

SOMATOMETRY OF BEAGLE DOGS

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The Beagle is the smallest of all scent hound breeds and the importance has always been stressed on the breed's conformation and its function. Because Beagles are very popular in the world there are often discussed two facts. The first that the breed has two standards and that there are certain types developing in geographically different areas with limited possibilities of new bloodline imports. Based on measuring 12 different body measurements (height at withers, length of back, loin, neck, chest circumference, length of foreleg, hock, second thigh, tail, ear and muzzle length and circumference) in a number of Beagles ranging from 147 to 115 individuals, it was discovered, that the often discussed problem of overgrowing is not justified, since the average height of 36.9 cm is not higher than the maximum of 40 cm as set by the standard. While closely studying measurements of body parts within a population bred by the AKC standard and the population of the KC standard, it was discovered that all body parts have a statistically significant difference. Even though an identical standard is used in the UK, continental Europe and in Australia and New Zealand, results have shown that there are statistical differences in height at withers between populations. The population of Australia and New Zealand has proved to have the lowest wither height which confirms observations of Musladin *et al.* (1998). A regression and correlation analysis has indicated that there is a significant dependence of length of back, chest circumference, length of foreleg, second thigh and tail as well as muzzle length and circumference on height at withers. The possibility of predicting adult height from puppy height at 6.5 weeks has shown only a mild dependence. Due to the fact that the prediction system has been proven in 50.65 % cases and disproven in 49.35 % it is impossible to confirm the hypothesis that it is possible to predict types and sizes of beagles from puppy measurements.

Adamson E: Tracking the Beagle's history. *All About America's Favorite Dogs: Beagles,* 05 (5), 5-13, 2005.
Didier A: Who's to blame? *The Beagle Annual.* Hoflin Publishing, Wheat Ridge, 1997, pp 98-99.
Dills J: The Jean Dills system of evaluating puppies. *Show Beagle Quarterly*, 13 (2), 14, 2007.
Dostál J: *Genetika a šlechtění psů.* Dona, České Budějovice, 2007. ISBN 9788073221041
Kitchell C: The importance of bite in the beagle. *Show Beagle Quarterly*, 15 (1), 21-54, 2009.
Kitchell C: Standards & ideals. *Show Beagle Quarterly,* 16 (1), 17-45, 2010.
Manifold B, Robblee S, Gilmore A: The form and function of a Beagle: movement under The American Beagle Standard. *Show Beagle Quarterly* 11 (4), 23-62, 2006.
Musladin JM, Musladin AC, Lueke AT: *The New Beagle*. Howell Book House, New York, 1998, p 256. ISBN 0876050283
Pavia A: Beagle essence. *All About America's Favorite Dogs: Beagles*, 98 (5), 12-23, 1998.
Phillips D: Predicting adult wither heights. *Show Beagle Quarterly,* 10 (3), 23-24, 2004.

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POSSIBILITIES OF ENVIRONMENTAL ENRICHMENT OF MACAQUES IN LABORATORY BREEDINGS AND ZOOS

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An old family of macaques come from Africa, then they settled in Asia, where they appear in the area from the seashore up to the altitude of 3 000 m where they live in snow for a period of the year. Therefore, when breeding them, we have to know what conditions they lived in when it comes to the research, top cross or raising them as a hobby. We have to lay great emphasis on selection of workers and breeders. It is important to provide safety and health aspects to these primates. They belong to the family of guenon monkey. They are narrow-nosed monkeys. Macaques live in couples, families or groups. They have large intra species differences; on that account we have to respect their environmental diversity and variability. The macaques are fed minimum twice a day. They live on fruit, seeds, blossoms, leaves, outgrowths, small animals, lizards, etc. The monkey should be kept in a separate building not to be bothered and not to disturb other dwellers of the ZOO. The pavilion must have perfect air-conditioning, heating and smooth, washable walls together with various systems of grabbing cages. The ZOO is only a substitution, as the space and possibilities are limited. The maintenance of the accurate biotope is difficult. Last but not least, there are economic expenses. The monkey needs society to its life. If the animal lives without other animals, it leads to its behaviour disorder, depression and self-destruction. The animal needs to live in a group. It should not be kept in a cage for the risk of aggression. The macaques spend great deal of the day half in the treetops. They seek trees for the night rest or as an escape against predators. In the run, the animals should have a small tree or branches that must be fixed enough not to hurt to them. They spend the rest of the day on the ground. The lack of flight to the top trees may cause fear and anxiety. Social aspects influence fertility of the animals, especially at the kind of primates living in groups. The primates compete with each other for space, roost, they range into pecking order. The animals necessarily need movement; we have to prevent their fattening and boredom. The floor should be covered by straw, excelsior, crushed paper, sawdust particles and soil. It is good to perform interaction between the man and the monkey for different periods of time. The interaction must be focused on understanding of sounds and visual perceptions that are specific for each species. There is a certain risk 6 of infection and injury, too. Some infections can be transmitted bi directionally. We have to be aware that the animals have a good memory and observe behaviour of humans towards them. Therefore we have to comply with the safety of the animals but also with our own one to prevent any trauma.

PARASITES OF GIBBONS *(HYLOBATIDAE)* IN HUMAN CARE

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We focused on mapping of parasites occurring in gibbons intestines in selected zoos all over the Czech Republic. Faeces analysis was conducted of 38 faecal specimens belonging to 4 species (*Nomascus gabriellae, Nomascus leucogenys, Hylobates lar, Symphalangus syndactylus*) from ZOO Liberec, ZOO Jihlava, ZOO Zlín – Lesná, ZOO Usti nad Labem, ZOO Olomouc, ZOO Ostrava, ZOO Plzen, ZOO Praha

and ZOO Chleby. Faeces were collected fresh and preserved in formaldehyde. Based on literature in the field, I determined a list of parasites which could occur and compared the list with actual findings. Faeces analysis was performed by sedimentation and flotation techniques and the results were evaluated using Statgraphics. For each individual gibbon, there is a summary of their health and anamnesis, equipment of quarters and care provided, as well as diet and daily routine enclosed. Three hypotheses were defined: the first assumed that the species composition of endoparasite gibbons is richer in juveniles than in adults. This hypothesis was not confirmed. The total number of parasites was, however, higher in individuals up to nine-years. The second hypothesis alleged that gender does not affect the species composition of endoparasite. This hypothesis was confirmed, but the total number of parasites was higher in males than in females. The third hypothesis stated that the type of gibbon does not affect the species composition of parasites. This hypothesis was confirmed as well. The total number of parasites was higher in *Nomascus leucogenys* and *Hylobates lar* species. However in zoos in the Czech Republic there are too few individuals of each species, so further research is encouraged. Our zoos do not use antiparasitics to prevent parasitism and only 8 subjects out of 38 were completely negative, therefore I propose to consider using antiparasitics.

Carpenter CA: Field study in Siam of the behaviour and social relations of gibbons (Hylobates lar). *Comparative Psychology Monographs*, 16, 1-201, 1994.
Fowler ME, Miller RE: *Zoo and Wild Animal Medicine, 5th edition.* 2003, pp 372, 376-377.
Holečková D, Dousek J: *Podmínky chovu savců volně žijících druhů v zajetí. Doporučení ÚKOZ.* Ministerstvo zemědělství, ARPA, Dvůr Králové nad Labem, 2000.
Levecke B et al.: Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. *Veterinary Parasitology*, 148, 236-246, 2007.
Muangkram Y et al.: A survey of intestinal parasites in gibbons at Krabokkoo Breeding Center Chachoengsao Province, Thailan, Chulalongkorn Uni. Fac. of Vet. Sc., pp 26-29, 2006.
O´Donoghue PJ et al.: New host record for the entodiniomorphid ciliate, Troglodytella abrassarti, from siamangs (Hylobates syndactylus). *Int J Parasitol*, 23 (3), 415-418, 1993.
Rowe N et al.: *The Pictorial Guide to the Living Primates*. Pogonias Press, Rhode Island, 1996. ISBN: 0-9648825-1-5
Redmond I: *Primates of the World*. New Holland Publisher, 2008, p 176.
Taylor MA et al.: *Veterinary Parasitology*. Wiley-Blackwell, 2007, p 600.
Volf P, Horák P et al.: *Paraziti a jejich biologie*. Triton, Praha, 2007. ISBN: 978-80-7387-008-9
Zajac AM, Conboy GA: *Veterinary Clinical Parasitology*. Blackwell Publishing, 2006, p 320.

PLAYING AND SOCIAL BEHAVIOUR OF HANUMAN LANGURS *(SEMNOPITHECUS ENTELLUS)*

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The main goal of this work is to describe social and playing behaviour of Hanuman langur species *(Semnopithecus entellus)*. The species of genus *Semnopithecus* are primates living in the biggest and most ecological diversificated area. They live all over the subcontinent of India and in every kind of ecosystems among evergreen forests. Social behaviour of primates means social contacts, group behaviour including grooming and presentation of anogenital area to other individuals in group, when their social hierarchy is formed. Langur's group size and social composition is commonly different. In articles are documented groups that have from 11 to 64 members. These groups are one-male, age graduating and multi-male. Hanuman langurs groups are formed from female lines and consolidation of females with one or more adult males. Adult, maturate and juvenile males who are not parts of multi-male groups, live solitary or in all-male groups. Social structure is formed due to interactions of individual strategies in society. Some authors consider play a specific form of learning. Other authors consider play as only as learning qualification. Play is formed in animals with high fylogenetic evolution level which have advanced central nervous system. Following ethogram is combination between diagnosticated models of behaviour and procedures which applicated different authors. This ethogram is published by Petru *et al.* (2009).

Agoramoorthy G: Primates in the Thar Desert. In: *Faunal Ecology and Conservation of the Great Indian Desert*. Springer-Verlag, Berlin, 2009, pp 177-191. DOI: 10.1007/978-3-540-87409-6_14
Borries C et al.: Males as infant protectors in hanuman langurs (*Presbytis entellus)* living in multimale groups – defence pattern, paternity and sexual behaviour. *Behav Ecol Sociobiol*, 46, 350-356, 1999.
Campbell CJ et al.: *Primates in Perspective*. Oxford University Press, Inc., 2007.
Dobroruka LJ: *Zvířata celého světa – 5, Poloopice a opice*. Státní zemědělské nakladatelství, Prague, 1979.
Chetan KSN et al.: Taxonomic implications of a field study of morphotypes of hanuman Langur *(Semnopithecus entellus)* in India. *Int J Primatol*, 2010.
Chhangani AK, Surendra MM: Ranging behaviour of Hanuman langurs (*Semnopithecus entellus)* in three different habitats. *Primate Conservation*, 21, 171-177, 2006.
Koenig A, Borries C: Socioecology of Hanuman langurs: the story of their success. *Evolutionary Anthropology*, 10, 122-137, 2001.
Petrů M et al.: Revisiting play elements and self-handicapping in play: a comparative ethogram of Old World monkey species. *J Comp Psychol*, 123, 250-263, 2009.
Sayers K, Norconk MA: Himalayan *Semnopithecus entellus* at Langtang national park, Nepal: diet, activity patterns, and resources. *Int J Primatol*, 29, 509-530, 2008.
Vasudev D et al.: Resource distribution and group size in the common langur *Semnopithecus entellus* in Southern India. *Am J Primatol*, 70, 680-689, 2008.

SAFETY OF COSMETIC COLORANTS EVALUATED BY ALTERNATIVE METHODS

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A number of colorants expected to be safe is widely used in consumer products, foods and feeds. Pigments and colorants approved for cosmetics are subject to exclusive regulation in the Cosmetics Directive 76/768/EEC, Annex IV. However, the available information on their safety is largely incomplete, sometimes confusing or controversial. The extent of data frequently does not comply with requirements of the SCCP´s Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation (2006). Our study was focused on the crucial missing toxicological data related to local toxicity of selected colouring agents allowed in all cosmetic products, in particular those regularly incorporated in leave-on products applied on skin areas exposed to sunlight. Colorants are obvious photoabsorbers in the UV/vis region and therefore phototoxicity assessment should be the prerequisite of their safe use in consumer products, particularly cosmetics. In a group of selected cosmetic colorants, 4 out of 13 substances exhibited phototoxic effect identified by means of *in vitro* 3T3 NRU Phototoxicity Test. The positive results were confirmed for 3 colorants by a phototoxicity assay on reconstructed human epidermis model (EpiDerm^{TM}), reflecting the intended topical skin exposure. However, no phototoxic effects were recorded using the human photopatch test suggesting that the bioavailable amount of penetrating substance does not reach the level capable to elicit a photodynamic reaction *in vivo*. Colorants exhibiting positive phototoxicity *in vitro* were subjected to further evaluation regarding skin penetration and possible mutagenic effects. The employed assays comprised evaluation of skin absorption *in vitro* on porcine skin, reverse mutation test using bacteria (Ames test) and *in vitro* mammalian chromosome aberration test. DNA fragmentation after irradiation of NIH3T3 cells was studied by means of single cell gel electrophoresis *in vitro* (comet assay). Although maximum of the applied dose of the substances remained on the skin surface and in the horny layer, a substantial proportion (up to 2.7 %) penetrated into the deeper skin layers and thus may elicit toxicologically significant adverse effects in metabolically active skin layers *in vivo*. In the Ames

test, a concentration dependent increase in revertant numbers, but never reaching the double, was observed in strain TA 102, both with/without S9 metabolic activation. The *in vitro* mammalian chromosome aberration test revealed a concentration dependent increase in the percentage of cells with chromosomal aberrations in case of all the 3 further investigated colorants, both with/without S9 metabolic activation. In the comet assay, a significant degree of DNA damage was detected after UVA irradiation in the highest test concentrations of the 3 tested colorants. Missing toxicity data for a number of allowed cosmetic colorants should be completed so that their possible hazard could be taken into account when safety assessment of finished cosmetic formulations is performed. Although used in low concentrations, the positive findings suggest that hazardous colorants exhibiting phototoxicity and/or mutagenicity potential may contribute to adverse skin reactions and/or body systems diseases, e.g. phototoxicity, photoallergy or even photocarcinogenicity.

PHOTOTOXICITY AND PENETRATION OF TIO_2 NANOPARTICLES DETERMINED BY ALTERNATIVE METHODS

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Project NANOCOVER, supported by Technological Agency of the Czech Republic, is focused on development of TiO_2 particles designed for specific applications (UV filters, antimicrobial functional textiles or photoactive self-cleaning coating materials). The phototoxic potential of TiO_2 particles was evaluated by means of the validated 3T3 NRU Phototoxicity Test and the results were confirmed using reconstructed human epidermis model Epiderm (MatTek, USA). Considering the crucial role of dermal penetration for systemic toxicity, five newly engineered TiO_2 nanoparticles (size 26-200 nm) were subjected to *in vitro* skin absorption/penetration studies in combination with sensitive analytical techniques and histological evaluation. Skin penetration was evaluated in compliance with OECD TG 428 using porcine skin *ex vivo*. Particles in various skin layers were determined by inductively coupled plasma mass spectrometry. Ultrathin sections of skin were evaluated using Transmission Electron Microscopy (TEM) to visualize the location of particles in individual skin layers. Reconstructed human epidermis (RhE) model EpiDerm was also assessed for skin penetration studies. The phototoxicity studies *in vitro* confirmed low cytotoxicity of the particles and absence of phototoxic potential up to the highest recommended test concentration of 1000 µg/ml. The skin absorption/penetration studies revealed that none of the tested TiO_2 particles penetrated into the receptor fluid. Regarding particles with size >100 nm, more than 95 % of the recovered TiO_2 remained on skin surface, about 4 % of TiO_2 was detected in stratum corneum/epidermis and less than 1 % in dermis. Regarding TiO_2 nanoparticles with size cca 26 nm in 0.5 % concentration, only 45 % of the recovered TiO_2 was found on skin surface, while cca 45 % of TiO_2 was found in stratum corneum/epidermis, with maximum accumulation in the first four layers of the stratum corneum, and 6 % in dermis. However, if penetration of TiO_2 particles sized 26 nm in 20.4 % concentration was assessed, the penetration pattern was similar to that of larger particles, tested in analogous high concentrations. TEM revealed penetration of TiO_2 of all sizes only up to the stratum corneum layer. In the cross section of hair follicle, nanoparticles of diameter 26 nm were found exclusively on the inner root sheath without any deeper penetration into stratum reticulare dermis and thus systemic exposure was excluded. The *in vitro* skin absorption method based on porcine skin is not able to discern substances diffusing along hairs into hair follicles, which may lead to false positive results in epidermal and dermal samples. Employment of RhE skin models without hairs may overcome limitations of the porcine skin.

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KUPFFER CELLS AMELIORATE HEPATIC INSULIN RESISTANCE INDUCED BY HIGH-FAT DIET RICH IN MONOUNSATURATED FATTY ACIDS: THE EVIDENCE FOR THE INVOLVEMENT OF ALTERNATIVELY ACTIVATED MACROPHAGES

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Resident macrophages (Kupffer cells, KCs) in the liver can undergo both pro- or anti-inflammatory activation pathway and exert either beneficiary or detrimental effects on liver metabolism. Until now, their role in the metabolically dysfunctional state of steatosis remains enigmatic. Aim of our study was to characterize the role of KCs in relation to the onset of hepatic insulin resistance induced by a high-fat (HF) diet rich in monounsaturated fatty acids. Male Wistar rats were fed either standard (SD) or high-fat (HF) diet for 4 weeks. Half of the animals were subjected to the acute GdCl3 treatment 24 and 72 hrs prior to the end of the experiment in order to induce the reduction of KCs population. We determined the effect of HF diet on activation status of liver macrophages and on the changes in hepatic insulin sensitivity and triacylglycerol metabolism imposed by acute KCs depletion by GdCl3. We found that a HF diet rich in MUFA itself triggers an alternative but not the classical activation program in KCs. In a steatotic, but not in normal liver, a reduction of the KCs population was associated with a decrease of alternative activation and with a shift towards the expression of pro-inflammatory activation markers, with the increased autophagy, elevated lysosomal lipolysis, increased formation of DAG, PKCε activation and marked exacerbation of HF diet-induced hepatic insulin resistance. We propose that in the presence of a high MUFA content the population of alternatively activated resident liver macrophages may mediate beneficial effects on liver insulin sensitivity and alleviate the metabolic disturbances imposed by HF diet feeding and steatosis. Our data indicate that macrophage polarization towards an alternative state might be a useful strategy for treating type 2 diabetes.

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MELATONIN APPLICATION IN BALB/C MICE WITH TULAREMIA

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Melatonin is a hormone with antioxidant properties. In the organism, melatonin is participated in regulation of circadian biological rhythm. But receptors for melatonin are expressed on disparate organs and they can be found on immune cells as well. This experiment is focused on research whether melatonin would regulate pathogenesis caused by a model intracellular pathogen, *Francisella tularensis*. Laboratory mice BALB/c were infected with *F. tularensis*. Melatonin was given in two doses: 10 and 100 µg/kg. Animals were sacrificed after either three or five days. Spleen and liver were sampled for bacterial burden. Interferon gamma (IFN-γ), interleukin 6 (IL-6) and total immunoglobulins were assayed from plasma samples. We verified that melatonin is able to reduce bacterial burden in the organs in a dose response manner. Surprisingly, IFN-γ and IL-2 levels were reduced as well. Immunoglobulins remained unchanged. We conclude our experiment that melatonin is potent to reduce tularemia progression. We deduce that the effect of melatonin lay in regulation of cell cycle rather than immune system regulation.

BENEFITS AND RISKS OF PROBIOTICS FOR IMMUNOCOMPROMISED NEWBORNS: A STUDY IN EXPERIMENTAL MODELS

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The gastrointestinal tract of mammalian newborns is sterile but it becomes colonized immediately after birth: babies delivered vaginally are colonized with organisms mainly from the mother's vaginal and intestinal microbiota whereas those delivered by Caesarean section are mainly colonized with environmental microbes. Infants born through Cesarean section had lower numbers of bifidobacteria and Bacteroides, whereas they were more often colonized with *Clostridium difficile*, compared with vaginally born infants. Exclusively formula-fed infants were more often colonized with *Escherichia coli*, *C. difficile*, Bacteroides, and lactobacilli, compared to breastfed infants. Probiotic properties have been ascribed to many microbial species, but those most commonly used are members of the lactic acid bacteria group, particularly *Lactobacillus* spp. and *Bifidobacterium* spp. A beneficial effect of probiotic therapy on a host health has been generally accepted. A situation in the case of immunocompromised preterm newborns is more complicated and using of probiotics in these cases has been disputed. Premature preterm born neonates lack an effective skin barrier, have an immature and often ineffective immune system, and quite often required prolonged support and hospitalization. Reduced digestive functions, motility and underdeveloped intestinal barrier in these newborns result in prolonged exposition of the intestine to different noxes and increased bacterial adherence, which predisposes to excessive fermentation, bacterial overgrowth and bacterial translocation. It may lead to overwhelming multisystem organ failure and death from systemic sepsis. Additional new information dealing with action of commensal and probiotic bacteria and their safety for immunocompromised preterm infants verified on suitable animal models has been required.

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ALTERNATIVE METHOD FOR ASSSESSMENT OF SKIN SENSITIZATION: NON-RADIOLABELLED LOCAL LYMPH NODE ASSAY LLNA:DA

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Allergic contact dermatitis is an important environmental and occupational health concern. It develops in two phases, induction and elicitation. Induction occurs when the sensitizing substance passes through epidermis, forms a hapten complex with dermal proteins which is processed by Langerhans cells and migrates to the draining lymph nodes. Antigen presentation to T-lymphocytes follows, leading to clonal expansion of these cells. The elicitation phase occurs when the individual is again topically exposed to the substance, which penetrates the skin and after procession by Langerhans cells is presented to the circulating T-lymphocytes. The antigen-specific T-lymphocytes are then activated, cause release of cytokines and other inflammatory mediators. Historically, the sensitizing potential of chemicals, medical devices and other consumer products has been evaluated by guinea pig test (GPT) methods (OECD TG 406, Guinea Pig Maximization Test GPMT and Bühler Test). However, due to ethical and scientific reasons, a substitute of this test, the Local Lymph Node Assay (LLNA) has been validated and adopted in 2002 (OECD TG 429). The basic principle underlying the traditional LLNA is that chemical sensitizers induce a primary proliferation (induction phase) of lymphocytes in the lymph nodes draining the site of chemical application which can be quantified using measurement of radio-labelled thymidine incorporation into the lymph node cellular DNA. This proliferation is proportional to the dose and potency of the sensitizer. The newly validated LLNA:DA, in comparison to the traditional LLNA, is based on the use of measuring ATP content, known to correlate to the living cell number, by a bioluminiscence method and thus eliminates the potential for occupational exposure to radioactivity and waste disposal issues. The LLNA provides a quantitative endpoint, dose-responsive data and allows for prediction of potency. GPT, which evaluates the elicitation phase of skin sensitization, provides a qualitative endpoint which tends to be highly variable in part due to its subjective nature (visual scoring of erythem/oedema). In comparison with the guinea pig tests, the LLNA:DA test procedure uses a reduced number of animals (mice, only 4 animals per group) and a shorter test period (8 days vs. min. 4 weeks in GPT). The stress and injury of the animals is considerably minimized (4 applications of 25 µl on both ears, killing of animals and dissection of lymph nodes on day 8, vs. intradermal induction with Freund´s adjuvant, epidermal induction and challenge connected with repeated handling and stress in GPMT). A further reduction of animals can be achieved by using a reduced version, when only the highest soluble dose is administered and the positive control group is not used in each test. Certain limitations of the LLNA:DA test have been identified (e.g. false negative results with certain metals) and the test is not appropriate for testing substances acting as ATP inhibitors or containing ATP degrading enzymes. Following an extensive validation study including 46 test substances, the LLNA:DA has been scientifically validated and legally accepted (OECD Test Guideline 442A Skin Sensitization: Local Lymph Node Assay: DA, 2010).

THE EFFECT OF RESVERATROL ON BONE METABOLISM IN RATS WITH BILE DUCT OBSTRUCTION

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We studied the influence of bile duct obstruction (BDO) after 28 days on bone metabolism status and their modification by resveratrol in male Wistar rats. The rats were divided into 3 groups: Sham group with laparotomy and vehiculum application, group BDO – bile duct obstruction and vehiculum application, and group R-BDO – bile duct obstruction and resveratrol application (10 mg/kg dose of resveratrol orally once daily). The bone mineral density (BMD; g/cm^2) and body composition were measured by dual energy X-ray absorptiometry. The physical strength of femur was examined by controlled break biomechanical testing. The osteocalcin, procollagen type I N-terminal propeptide (PINP) and carboxy-terminal collagen crosslinks (CTX) in the bone tissue homogenate were analysed by EIA. The net BMD was lower in group BDO (0.176±0.005) and R-BDO (0.181±0.004) vs. Sham (0.209±0.003). Body fat (g, median) was lower in BDO (1.90) and R-BDO (2.55) than in Sham (3.45). The force needed for fracture of femurs (N) significantly decreased in BDO (154±6) and R-BDO (151±13) vs. Sham group (208±7). The force needed for neck fracture decreased in BDO (105±3) and R-BDO (115±8) vs. Sham (135±9). The osteocalcin decrease in group R-BDO (2.9±0.1) vs. Sham (3.7±0.4). Higher PINP/CTX were in BDO (20±5/0.49±0.08) and R-BDO (30±4/0.55±0.07) vs. Sham (16±4/0.48±0.11). In conclusions, our findings suggest that subchronic bile duct obstruction decreased body fat quantity and BMD with decrease of bone rigidity. Resveratrol showed positive effect on liver injury and consequently rendered mild positive influence on bone impairment.

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MODULATION OF LIVER REGENERATION AFTER PARTIAL HEPATECTOMY BY DIETARY CHOLESTEROL IN RATS

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The aim of study was to evaluate impact of long-term dietary cholesterol overload on the cholesterol homeostasis and liver regeneration in rats. Serum lipid parameters, tissue ^{14}C-cholesterol incorporation, liver DNA synthesis and protein expression was determined in rats fed with a standard (SLD) or hypercholesterolemic (CHOL) diet and with partial hepatectomy (PH). 29-day continual intake of high cholesterol diet in rats before PH produced increase in serum total cholesterol, LDL lipoprotein, and triglyceride concentration. PH provoked decrease in serum total cholesterol, and triglyceride concentration in both SLD and CHOL groups. PH was associated with increase in serum ALT activity more pronounced in CHOL animals. DNA synthesis in regenerating liver was increased after PH in both dietary groups, but lower in CHOL animals. Importantly, hypercholesterolemic diet reduced the absorption of orally applied radiolabelled cholesterol in intestine and then activity in blood and liver. The ^{14}C-cholesterol hepatic activities tend to increase after PH in both diet groups. Long-term CHOL diet produced up-regulation of Acyl-CoA:cholesterol acyltransferase-2 protein expression. PH was associated with marked increase of LDL receptor and Acyl-CoA:cholesterol acyltransferase-2 protein expression in both dietary groups. In conclusions, decreased cholesterol absorption in GIT is suggested as compensatory mechanism during high cholesterol dietary intake. Importantly, high cholesterol diet in rats negatively influenced liver regeneration after PH. The increased uptake of cholesterol in the liver after PH which was associated with up-regulation of LDL receptor protein expression suggests preferential use of extrahepatic cholesterol by the liver under these critical conditions.

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