Literature Search Product

for
Urea

(CAS No. 57-13-6)

Prepared for the Oak Ridge Institute for Science and Education
In Support of the Toxicological Review by the
Integrated Risk Information System (IRIS)
Prepared by ILS, Inc.

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Search Strategy and Presentation of Results

The Statement of Work provided by ORISE requested a literature search for urea to identify sources of information to be considered for inclusion in Chapters 1-4 of the Toxicological Review. Searches of the biomedical databases for relevant toxicity, toxicokinetic and related information on urea were hampered by the large number of retrievals found due to urea being a natural metabolite and having a large number of uses, including chemical reactant in organic synthesis, fertilizer, additive to feeds, diagnostic for kidney failure, and drug for osmoregulation. In some databases, the names of urea derivatives were often fragmented so that urea is a separate word (e.g., methylurea as methyl urea or monomethyl urea). Even using "Urea/adverse effects" as a MeSH heading in MEDLINE retrieved records on derivatives even when the derivative name was written as one word.

PubMed and Google Scolar were initially browsed using the terms "urea", "57-13-6", and combinations of terms pertaining to adverse effects, toxicity, health effects, damage, etc., to identify concepts that could be excluded from the search strategy before fee-based searches were done. New concepts and terms were also noted when found. Several full articles and reviews were retrieved and examined for additional information that could be used in the search strategy to help limit retrievals. The 1993 JECFA file was reviewed as was the Cosmetic Ingredient Review Final Report (Yamarik and Elmore, 2005) and PUBMED records for relevant citations were retrieved.

Records found on TOXNET were printed from CCRIS, ChemIDplus, and HSDB along with Information from the PAN Pesticide Database, the Carcinogenic Potency Database, and the Substance Registry System (U.S. EPA). Selected records found on the Environmental Chemical Information System on BIBLIOLINE were also printed from NIOSHTIC and RISKLINE, along with the urea records in RTECS and TSCATS. Selected records were also retrieved from EMIC and DART.

STN International database files MEDLINE, CABA, AGRICOLA, EMBASE, ESBIOBASE, BIOTECHNO, IPA, BIOSIS, TOXCENTER, DISSABS, SCISEARCH, FROSTI, FSTA, PASCAL, and NTIS were searched simultaneously on July 2, 2007. Essentially, the strategy used the common synonyms and CAS Registry Number (CAS RN) for urea and deleted some names that might have been fragmented in the database record, leaving urea as a whole word, or that might have been indexed as urea compounds. A number of concepts, such as ruminant animals and hemodialysis, were excluded. Initially, a total 448,031 database records (including multiple duplicates in different databases) was reduced to 204,052 by this strategy. This set was then combined with terms that were desirable for the answer set and some additional terms were deleted giving 62,247 records. Further reduction to three smaller sets was accomplished through duplicate removal. The records in these three lists totaled approximately 15,000. Adding a requirement that the CAS RN must be in the record culled the list to < 4,000 records. Due to time constraints, the titles of 3,515 records (L66) that had urea in the title were examined. The edited history of the online session is presented below (some intermediate answer sets were inadvertently or intentionally omitted in the derivation of the ultimate answer sets so there are gaps in the L numbers).

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L1      151404 57-13-6
L2      447489 CARBAMIDE OR UREA OR CARBONYL(W) DIAMIDE
L3      448031 L1 OR L2
L4         397019 L3 NOT (FORMALDEHYDE OR DERIVATIV? OR UREA(W)(BASED OR COMPOUND?) OR
IMIDAZOLIDINYL? OR
               HYDROXYUREA OR NITROSO? OR NITROSAM? OR DIAZOLIDINYL?)
QUERY L4 HAS BEEN SAVED AS 'UREANAME/Q'
L5      371347 L4 NOT (INBORN(W) ERROR? OR HEMODIALY? OR HAEMODIALY? OR DIALY? OR MICRODIALY?)
L6      370571 L5 NOT UREA(W) CYCLE(W) DISORDER?
L7      364665 L6 NOT PATENT/DT
L8      334972 L7 NOT (RUMINANT? OR RUMEN OR GOAT? OR SHEEP OR LAMB?)
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L9      320593 L8 NOT (COW? OR CATTLE OR LIVESTOCK)
L10     320566 L9 NOT BISON
L12     316174 L10 NOT (BIRD? OR CHICKEN?)
L13     309464 L12 NOT (FISH? OR TELEOST? OR CAMEL?)
L14     309370 L13 NOT (DROSOPHILA AND (RESISTANCE OR TOLERANCE))
L15     276783 L14 NOT THERAP?
L16     276631 L15 NOT (HERITABLE(W) DISORDER? OR POLYDIPSIA)
L17     276452 L16 NOT (SATURNISM OR LEAD(W) POISONING)
L18     275960 L17 NOT (INTRAMNIOTIC OR TERMINAT?(3A) PREGNANCY OR ABORT?)
L19     275599 L18 NOT SICKL?
L20     268075 L19 NOT PYLORI
L21     267587 L20 NOT BLOOD(W) BRAIN(W) BARRIER
L22     213085 L21 NOT PLANT?
L23     209376 L22 NOT HERBICID?
L24     204958 L23 NOT (SKIN OR DERMA? OR PERCUTANEOUS? OR CONTACT(W) SENSIT?)
L27       7359 L24 AND (MALIGNAN? OR NEOPLAS? OR CANCER? OR CARCINO?)
L28       2228 L27 AND (RATS OR MICE)
L29        582 L24 AND CARCINOGENESIS
L30     204052 L24 NOT (BLEACH? OR WHITENING OR TEETH OR TOOTH)
L31       1180 L27 AND UREA/TI
L32       1139 L31 NOT UREA(W) CYCL?
L33        342 L32 AND (RATS OR MICE)
L34        933 L30 AND (INHAL? OR INTRATRACHEAL? OR ENDOTRACHEAL?)
L35        435 L34 AND UREA(W) NITROGEN
L36         30 L34 AND UREA/TI
L38         27 L36 NOT L35
L39         27 L34 AND WORKER?
L42         54 L38 OR L39
L43       4982 L30 AND (CHROMOSOM? OR MUTAGEN? OR MICRONUCLE? OR L5178Y OR SISTER(W) CHROMATID)
L44         44 L30 AND (ANEUPLOID? OR POLYPLOID?)
L46       8131 L30 AND (CHRONIC? OR SUBCHRONIC? OR REPEATED(W) DOSE)
QUERY L30 HAS BEEN SAVED AS 'UREANOT/Q'
L47       2852 UREA/TI AND L45
L48      16206 L33 OR L42 OR L43 OR L44 OR L46 OR L47
L49      25944 L30 AND (TOXIC? OR GENOTOXIC? OR TERATOGEN? OR EMBRYO? OR FOETUS OR FETUS OR
FETAL OR FOETAL
               OR FETO? OR FOETO?)
L50      25672 L30 AND (LONG(W) TERM OR MG(W) KG OR DOSE? OR DOSING OR DOSAGE OR MULTIDOS?)
L51      32885 L30 AND (CLEARANCE OR RATE OR DOSE(W) RESPONSE OR LUNG OR LUNGS OR PULMONARY)
L52       8174 L30 AND (INTOXICA? OR TRANSFORM? OR MALFORM? OR EXOGENOUS?)
L53       1379 L30 AND (SUDDEN(W) INFANT(W) DEATH OR OXIDATIVE(W) STRESS)
L54      76747 L48 OR L48 OR L50 OR L51 OR L52 OR L53
L55      62380 L54 NOT (TRANSPORT? OR UREA(W) STRESS OR ACID(W) BASE(W) BALANCE OR CULTURE? OR
DIABET?)
L56      62247 L55 NOT (NITROGEN(W) SALVAG? OR OSMOREGULAT?)
L57      10539 L56 AND UREA/TI
L58      51708 L56 NOT L57
L59      10231 L58 AND UREA/CT (Not a valid field code for three databases.)
L60      41477 L58 NOT L59
L61       4797 L60 AND 57-13-6
L62       5489 L57 AND 57-13-6
L63       9632 L59 AND 57-13-6
L64      19918 L61 OR L62 OR L63
L65       3962 DUP REM L61 (835 DUPLICATES REMOVED)
L66       3515 DUP REM L62 (1974 DUPLICATES REMOVED)
L67       7436 DUP REM L63 (2196 DUPLICATES REMOVED)
L68       3962 DUP REM L65 (0 DUPLICATES REMOVED)
L69      42329 L56 NOT L64
ANSWER SET L69 HAS BEEN SAVED AS 'UREASELREST/A'
ANSWER SET L65 HAS BEEN SAVED AS 'UREA2/A'
ANSWER SET L66 HAS BEEN SAVED AS 'UREA3/A'
ANSWER SET L67 HAS BEEN SAVED AS 'UREA4/A'
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A total of 352 records were selected from the 3,515 titles and retrieved in full format. They were obtained from MEDLINE (216), BIOSIS (89), TOXCENTER (18), EMBASE (16), CABA (11), and AGRICOLA (2). TOXCENTER is made up of the databases comprising TOXLINE plus CAPLUS. Since CAPLUS had already been searched, the selections from TOXCENTER were lower than expected for a multi-file search such as this.

CAPLUS (Chemical Abstracts) records are more reliably indexed with the CAS RN than were other records. Urea's CAS RN was linked with the indexing term for "adverse effects" in 578 records. Because 190 of these had already been selected from PubMed searches, only 33 CAPLUS records were selected from the record titles for retrieval in full format. The bibliogry for the references retrieved for further review along with those selected online (589 references total) are in the accompanying EndNote file.

The following Literature Search Product presents 383 references from STN and the 206 references from PubMed and online sources; of these ~250 are highlighted for primary consideration in the Toxicological Review and are organized according to the IRIS 2006 Toxicological Review template. Some references are presented in 2 or more different sections when they appear applicable. In Section 3, a few references are placed in an initial general section because they refer to more than one of the categories Uptake, Distribution, Metabolism, Excretion, or PBPK. Abstracts were included only with the first listing.

Secondary material (review articles, reports, or overviews from other agencies) applying to several or all of Sections 2–4.

BUA-Stoffbericht. (1995) **Urea; isobutylidene diurea; potassium amylxanthate; potassium isobutylxanthate**. Stuttgart, Germany: S. Hirzel. OCLC No. 33099404.

Cosmetic Ingredient Review. (2005) **Final report of the safety assessment of urea**. Int J Toxicol 24: 1-56. Although Urea is officially described as a buffering agent, humectant, and skin-conditioning agent-humectant for use in cosmetic products, there is a report stating that Urea also is used in cosmetics for its desquamating and antimicrobial action. In 2001, the Food and Drug Administration (FDA) reported that Urea was used in 239 formulations. Concentrations of use for Urea ranged from 0.01% to 10%. Urea is generally recognized as safe by FDA for the following uses: side-seam cements for food contact; an inhibitor or stabilizer in pesticide formulations and formulations applied to animals; internal sizing for paper and paperboard and surface sizing and coating of paper and paper board that contact water-in-oil dairy emulsions, low-moisture fats and oils, moist bakery products, dry solids with surface containing no free fats or oil, and dry solids with the surface of fat or oil; and to facilitate fermentation of wine. Urea is the end product of mammalian protein metabolism and the chief nitrogenous compound of urine. Urea concentrations in muscle, liver, and fetuses of rats increased after a subcutaneous injection of Urea. Urea diffused readily through the placenta and into other maternal and fetal organs. The half-life of Urea injected into rabbits was on the order of several hours, and the reutilization rate was 32.2% to 88.8%. Urea given to rats by a bolus injection or continuous infusion resulted in distribution to the following brain regions: frontal lobe, caudate nucleus, hippocampus, thalamus plus hypothalamus, pons and white matter (corpus callosum). The permeability constant after treatment with Urea of whole skin and the dermis of rabbits was 2.37 +/- 0.13 (x 10(6)) and 1.20 +/- 0.09 (x10(3)) cm/min, respectively. The absorption of Urea across normal and abraded human skin was 9.5% +/- 2.3% and 67.9% +/- 5.6%, respectively. Urea increased the skin penetration of other compounds, including hydrocortisone. No toxicity was observed for Urea at levels as high as 2000 mg/kg in acute oral studies using female rats or mice. No signs of toxicity were observed in male piglets dosed orally with up to 4 g/kg Urea for 5 days. Dogs dosed orally with 5 to 30 g/L Urea for 4 to 10 days had signs of toxicity, including weakness, anorexia, vomiting and retching, diarrhea and a decreased body temperature, which led to a deep torpor or coma. No significant microscopic changes were observed in the skin of male nude mice dermally exposed to 100% Urea for 24 h. No observable effect on fetal development was seen in rats and mice dosed orally with an aqueous solution of Urea (2000 mg/kg) on days 10 and 12 of gestation. The mean number of implants, live fetuses, percent fetal resorptions, mean fetal weight, and percent fetuses malformed were comparable to control group. A detergent containing 15% Urea was injected into pregnant ICR-JCl mice and dams and fetuses had no significant differences when compared to control animals. Urea given orally did not enhance the developmental toxicity of N-nitrosomethylurea. Female Sprague-Dawley rats injected in the uterine horn with 0.05 ml Urea on day 3 (preimplantation) or on day 7 (post implantation) exhibited no maternal mortality or morbidity; a dose-dependent reduction in embryo survival was seen with preimplantation treatment. Urea injected intra-amniotically induces mid-trimester abortions in humans. Urea was not genotoxic in several bacterial and mammalian assays; although in assays where Urea was used at a high concentration, genotoxicity was found, many in in vitro assays. Urea is commonly used in studies of DNA because it causes uncoiling of DNA molecules. Urea was not carcinogenic in Fisher 344 rats or C57B1/6 mice fed diets containing up to 4.5% Urea. Exposure of normal human skin to 60% Urea produced no significant irritation in one study, but 5% Urea was slightly irritating and 20% Urea was irritating in other reports. Burning sensations are the most frequently reported effect of Urea used alone or with other agents in treatment of diseased skin. Overall, there are few reports of sensitization among the many clinical studies that report use of Urea in treatment of diseased skin. The Cosmetic Ingredient Review (CIR) Expert Panel determined the data provided in this

report to be sufficient to assess the safety of Urea. The Panel did note that Urea can cause uncoiling of DNA, a property used in many DNA studies, but concluded that this in vitro activity is not linked to any in vivo genotoxic activity. Although noting that formulators should be aware that Urea can increase the percutaneous absorption of other chemicals, the CIR Expert Panel concluded that Urea is safe as used in cosmetic products.

Izmerov, NF. (1988) **Urea – herbicides**. Moscow, USSR: Centre for International Projects, GKNT, 52 pp. OCLC No. 40779213.

NLM (National Library of Medicine). **Urea**. Chemical Carcinogenesis Research Information System (CCRIS). National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

NLM (National Library of Medicine). **Urea**. Hazardous Substances Data Bank (HSDB). National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

JECFA. (1993) **Urea**. WHO Food Additives Series 32: 253-263. Geneva, Switzerland: World Health Organization. Available from: <http://www.inchem.org/documents/jecfa/jecmono/v32je16.htm>. Since urea is a natural end-product of amino acid metabolism in humans, and that approximately 20 grams/day is excreted in the urine in adults (proportionately less in children) the Committee concluded that the use of urea at levels of up to 3 % in chewing-gum was of no toxicological concern.

Juszkiewicz, T. (1966) **The toxi-city of urea. Original Title: Toksycznosc mocznika**. Med Weter 22: 65-68.

Richet C; Maret R. (1951) **[Toxicity of urea]**. Bulletin de l'Academie nationale de medecine, 135: 21-22.

RTECS (Registry of Toxic Effects of Chemical Substances). **CAS Registry Number: 57-13-6. Chemical Name: Urea**. RTECS Number: YR6250000. Database provided by the BiblioLine Basic - Environmental Chemistry Information System (ECIS), National Information Services Corporation, Baltimore, MD.

TAP (Technical Advisory Panel). (2004). **Tap Report for Urea**. Available from: <http://www.ams.usda.gov/nop/NationalList/UreaFinal.pdf>.

Tracor-Jitco, I. (1974) **Scientific Literature Reviews on Generally Recognized as Safe (GRAS) Food Ingredients: Urea.** NTIS PB Report (PB-241 971), 207 pp.

U.S. EPA (Environmental Protection Agency). (1995) **Pesticide fact sheet urea.** Report No. EPA/737/F-95/015. Microfiche. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, 8 pp. OCLC No. 49571341. This document contains up-to-date chemical information, including a summary of the Agency's regulatory position and rationale, on a specific pesticide or group of pesticides. A Fact Sheet is issued after registration of a significantly changed pattern.

Reviews pertaining to endogenous urea and urea synthesis

Amtul, Z; Rahman, AU; Siddiqui, RA; et al. (2002) **Chemistry and mechanism of urease inhibition**. Curr Med Chem 9: 1323-1348. Studies on enzyme inhibition remain an important area of pharmaceutical research since these studies have led to the discoveries of drugs useful in a variety of physiological conditions. The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates. Urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs. Ironically, urease was the first enzyme crystallized but its mechanism of action is still largely misunderstood. This chapter therefore reviews comprehensive developments in the field of urease inhibitors. Inhibitors of urease can be broadly classified into two categories: (1) active site directed (substrate-like), (2) mechanism-based directed. We present here the examples of selected inhibitors along with their mechanisms of action to characterize their mode of urease inhibition. The observations that urease due to its high substrate (urea) specificity can only bind to a few inhibitors with a similar binding mode as urea is also discussed. Several non-covalent interactions including hydrogen bonds and hydrophobic contacts stabilize the enzyme-inhibitor complex. Regardless of the class of compound, it is reported that only a few functional groups with electronegative atoms such as oxygen, nitrogen and sulfur act either as bidentate (mostly), tridentate (rarely), or as ligand-chelator to form octahedral complexes with two slightly distorted octahedral Ni ions of the enzyme. Bulky groups attached to the pharmacophore were found to decrease the activity of inhibitors, since the lack of a bulky attachment makes it easier for urease inhibitors to enter the substrate-binding pocket as well as avoid unfavorable steric interactions with amino acid residues in its vicinity. This review is intended to provide highlights of the inhibition of urease by hydroxamic acids (HXAs), phosphorodiamidates (PPDs), imidazoles, phosphazene and related compounds. These compounds are compared to previously reported urease inhibitors for the catalytic models proposed for urease activity. The differences in inhibition of urease activities from plants and of bacterial origin by various inhibitors and physiological implications of urease inhibition are discussed.

Boure, T; Vanholder, R. (2004) **Biochemical and clinical evidence for uremic toxicity**. Artif Organs 28: 248-253. The uremic syndrome is a mix of clinical features resulting from multiple organ dysfunctions which develop when kidney failure progresses, and is attributed to the retention of solutes, which under normal conditions are excreted by the healthy kidneys into the urine. The most practical classification of uremic solutes is based on their physicochemical characteristics that influence their dialytic removal, in (1) small water soluble compounds, (2) the larger "middle molecules," and (3) the protein bound compounds. Hence, uremic retention is much more complex than originally believed. Among the small water soluble compounds, urea exerts not much toxic activity and is not very representative in its kinetic behavior for many other uremic solutes. Among the middle molecules, many have been recognized to exert biological activity and hence to contribute to the uremic syndrome. Specific dialysis strategies apply large pore membranes to remove those middle molecules and have a beneficial impact on uremic morbidity and mortality. A substantial number of uremic solutes are protein bound. Only recently, a relation between their concentration and clinical status could be demonstrated. Likewise, it was only recently possible to demonstrate more than standard removal with super-flux dialysis membranes. To further improve characterization of uremic solutes and to develop directed therapeutic approaches, further concerted action among various groups of researchers will be needed.

Aoyagi K. (2003) **Inhibition of arginine synthesis by urea: A mechanism for arginine deficiency in renal failure which leads to increased hydroxyl radical generation**. Mol Cell Biochem 244: 1-2. We have reported that (1) the synthesis of GSA, a uremic toxin, increases depending on the urea concentration and (2) GSA is formed from argininosuccinic acid (ASA) and the hydroxyl radical or SIN-1 which generates superoxide and NO simultaneously. However, an excess of NO, which also serves as a

scavenger of the hydroxyl radical, inhibited GSA synthesis. We also reported that arginine, citrulline or ammonia plus ornithine, all of which increase arginine, inhibit GSA synthesis even in the presence of urea. To elucidate the mechanism for increased GSA synthesis by urea, we investigated the effect of urea on ASA and arginine, the immediate precursor of NO. Isolated rat hepatocytes were incubated in 6 ml of Krebs-Henseleit bicarbonate buffer containing 3% bovine serum albumin, 10 mM sodium lactate, 10 mM ammonium chloride and with or without 36 mM of urea and 0.5 or 5 mM ornithine at 37(C for 20 min. In vivo experiments, 4 ml/100 g body weight of 1.7 M urea or 1.7 M NaCl were injected intra-peritoneally into 5 male Wistar rats. Two hours after the intra-peritoneal injection of urea or 1.7 M NaCl, blood, liver and kidney were obtained by the freeze cramp method and amino acids were determined by an amino acid analyzer (JEOL:JCL-300). ASA in isolated hepatocytes was not detected with or without 36 mM (200 mgN/dl) urea, but the arginine level decreased from 36 to 33 nmol/g wet cells with urea. Ornithine which inhibits GSA synthesis, increased ASA markedly in a dose dependent manner and increased arginine. At 2 h after the urea injection the rat serum arginine level decreased by 42% (n = 5), and ornithine and citrulline levels increased significantly. Urea injection increased the ASA level in liver from 36-51 nmol/g liver but this was not statistically significant. We propose that urea inhibits arginine synthesis in hepatocytes, where the arginine level is extremely low to begin with, which decreases NO production which, in turn, increases hydroxyl radical generation from superoxide and NO. This may, also, be an explanation for the reported increase in oxygen stress in renal failure.

Bianchi GP; Bolzani R; Marchesini G; et al. (1993) **Mathematical modelling of amino acid-derived urea synthesis**. Ital J Gastroenterol 25: 87-92.

Cohen PP. (1981) **The ornithine-urea cycle: biosynthesis and regulation of carbamyl phosphate synthetase I and ornithine transcarbamylase**. Curr Top Cell Regul 18: 1-19.

Dimski DS. (1994) **Ammonia metabolism and the urea cycle: function and clinical implications**. J Vet Intern Med 8: 73-78. Disposal of waste products accumulated during metabolic processes is integral to the health of any living organism. Disposal of excess nitrogen and ammonia is no exception. Although nitrogen is essential for growth and maintenance in animals, an excess of some nitrogenous compounds can quickly lead to toxicity and death. Because of the correlation between ammonia accumulation and clinical disease, it is important for veterinary clinicians to understand the physiological mechanisms used to dispose of nitrogen and ammonia. Therefore, the purposes of this article are to review ammonia metabolism, the urea cycle, and the clinical implications of urea cycle dysfunction in diseases of companion animals.

Freedland RA; Meijer AJ; Tager JM. (1985) **Nutritional influences on the distribution of the urea cycle: intermediates in isolated hepatocytes**. Fed Proc 44: 2453-2457. After the urea cycle was proposed, considerable efforts were put forth to identify critical intermediates. This was then followed by studies of dietary and nutritional control of urea cycle enzyme activity and allosteric effectors of urea cycle enzymes. Correlation of urea cycle enzyme activity with isolated cell experiments indicated conditions where enzyme activity would be rate limiting. At physiological levels of ammonia the activation of carbamoyl-phosphate synthetase (EC 6.3.4.16) by N-acetylglutamate (NAG) is important. Various levels of NAG corresponded well with changes in the rate of citrulline and urea synthesis. Arginine was found to be an allosteric activator of N-acetylglutamate synthetase (EC 2.3.1.1). Therefore, it was possible that the rate of carbamoyl phosphate synthesis was dependent on the level of urea cycle intermediates, particularly arginine. Evidence for arginine in the regulation of NAG synthesis is not as clear as for NAG on carbamoyl phosphate synthetase I. The concentration of hepatic arginine is not necessarily an indication of the mitochondrial concentration. Only mitochondrial arginine stimulates the N-acetylglutamate synthetase. Recent studies indicate that the mitochondrial concentration of arginine is

higher than the cytosolic concentration and is well above the Ka for N-acetylglutamate synthetase. Therefore, it appears that changes in arginine concentration are not physiologically important in regulating levels of NAG. However, it is possible that responses to the effector may vary with time after eating, and it may be this responsiveness that controls the level of NAG and thereby urea synthesis.

Grisolia S; Minana MD; Grau E; et al. (1993) **Control of urea synthesis and ammonia detoxification**. Adv Exp Med Biol 341: 1-12.

Jackson AA. (1995) **Salvage of urea-nitrogen and protein requirements**. Proc Nutr Soc 54: 535-547.

Jackson AA. (1998) **Salvage of urea-nitrogen in the large bowel: functional significance in metabolic control and adaptation**. Biochem Soc Trans 26: 231-236.

Raab W. (1989) **[Biochemistry, pharmacology and toxicology of urea]**. Hautarzt 40 (Suppl 9): 23-26. In modern dermatology urea has two areas of application: --Applied topically urea is a pharmacologically active substance. It is moisturizing, keratolytic (in high concentrations only), desquamating, antipruritic, and antimicrobial. --Urea supplements and increases the effectivity of glucocorticoids, anthralin (dithranol) and tretinion. Urea-glucocorticoid combinations deserve special attention.

Shambaugh GE. (1977) **Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle**. Am J Clin Nutr 30: 2083-2087. The urea cycle consist of five enzymatically controlled steps that are catalyzed by carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinase, and arginase, respectively. The complete cycle is present in physiological meaningful levels in the liver of terrestrial vertebrates, and in man represents the sole mechanism for ammonia disposal. The formation of carbamyl phosphate and the synthesis of argininosuccinate are potential limiting steps in urea biosynthesis but substrate and not enzymes levels are rate-limiting under physiological conditions. In the adult, urea cycle enzymes change as a unit, and are largely influenced by dietary protein content. The urea cycle is closely linked to the citric acid cycle deriving one of its nitrogens through transamination of oxalacetate to form asparate and returns fumarate to that cycle. The biosynthesis of urea demands the expenditure of energy but less than 20% of the energy derived from metabolism of gluconeogenic amino acids is required for ureogenesis. Embryological development of the urea cycle in the tadpole and in mammalian fetal liver therefore permits use of amino acids as new sources of energy to meet oxidative demands for continuing growth.

Steinbrecher HA; Griffiths DM; Jackson AA. (1992) **Urea production in normal breast-fed infants measured with primed/intermittent oral doses of [15N, 15N]urea**. Acta Paediatr 85: 656-662. Urea kinetics were measured on 11 occasions in six normal, breast-fed infants aged 29-88 days. Prime and intermittent oral doses of [15N, 15N]urea with measurement of enrichment of urea in urine were used. The rate at which urea appeared in the urea pool was 265 mgN/kg per hour, 85% of which derived from endogenous production and 15% from the diet. Urinary excretion of urea was 87 mgN/kg per hour. Therefore, 60% of the urea entering the pool each day was hydrolysed by the metabolic activity of the colonic microflora and the nitrogen was made available for further metabolic interaction. The rate of urea appearance and the extent to which urea nitrogen was salvaged were greater in infants under 6 weeks than in those over 6 weeks, indicating that urea kinetics is a more active process at an early age, and slows with time. With respect to factors influencing urea kinetics, the apparently conflicting results which have appeared in the literature may be explained. The results may help explain the growth of breast-fed infants on low protein intakes.

Urakabe S; Orita Y; Shirai D; et al. (1971) **Non-protein nitrogen (NPN) and blood urea nitrogen**

(BUN)--how to read the figures. Nippon Rinsho 29: 220-230.

Visek WJ. (1979) **Ammonia metabolism, urea cycle capacity and their biochemical assessment**. Nutr Rev 37: 273-282.

Visek WJ. (1972) **Effects of urea hydrolysis on cell life-span and metabolism**. Fed Proc 31: 1178-1193.

2. CHEMICAL AND PHYSICAL INFORMATION

IPCS (International Programme on Chemical Safety). (2001). **Urea**. ICSC: 0595. Available from: <http://www.inchem.org/documents/icsc/icsc/eics0595.htm>.

NIAID (National Institute of Allergy and Infectious Disease). (undated) **Urea**. National Institutes of Health. Availabe from: <http://chemdb.niaid.nih.gov/struct_search/default.asp>.

NLM (National Library of Medicine). **Urea**. ChemIDplus Advanced. National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

NLM (National Library of Medicine). **Urea**. PubChem. National Center for Biotechnology Information.. National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

OECD SIDS (Organisation for Economic Co-operation and Development Screening Information Data Set). (undated). **Urea. CAS N^{o}: 57-13-6**. Available from: <http://www.inchem.org/documents/sids/sids/57136.pdf>.

O'Neil, MJ; Heckelman, PE; Koch, CB; et al., eds. (2006) **The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals**. 14th ed. Whitehouse Station, NJ: Merck and Co., Inc.; p. 9867.

Registry. 2007. **RN 57-13-6**. Database produced by Chemical Abstract Service (CAS) and available on STN.

3. TOXICOKINETICS

Ahloulay M; Dechaux M; Laborde K; et al. (1995) **Influence of glucagon on GFR and on urea and electrolyte excretion: direct and indirect effects**. Am J Physiol 269: F225-35. Clearance experiments were performed in anesthetized male Wistar rats to determine the level of peripheral glucagon concentration required to elicit changes in glomerular filtration rate (GFR) and in solute excretion. Glucagon was intravenously infused at a rate of 1.25 (group G-1, n = 8), 3.75 (group G-3, n = 7), or 12.5 (group G-10, n = 7) ng.min-1.100 g body wt-1 for 100 min. Measurements were performed before, during, and after this infusion. Group G-10 resulted in a plasma concentration of glucagon severalfold higher than usually observed in peripheral blood after a protein meal but normal for the hepatic circulation. Group G-10 simultaneously increased GFR, plasma adenosine 3',5'-cyclic monophosphate (cAMP) concentration, and the excretion of water (i.e., urinary flow rate), Na, Cl, PO4, K, and urea. Some of the effects of glucagon on electrolyte excretion were also observed with group G-1 and/or G-3 and were fully reversible, suggesting a direct renal action of glucagon. The significant and reversible increase in K excretion in group G-3 suggests that glucagon exerts a direct stimulatory influence on K

secretion in the distal nephron. Increases in urinary flow rate, PO4, Na, and urea fractional excretions were seen with group G-10 only and were not reversible, suggesting an indirect action of glucagon on the proximal tubule. Because glucagon stimulates cAMP formation in hepatocytes and because this cAMP is released in the blood and secreted by proximal tubule cells, cAMP of hepatic origin could induce a parathyroid hormone-like effect in this nephron segment. In summary, these experiments suggest that glucagon influences different aspects of renal function by a combination of direct and indirect (probably liver-dependent) effects.

Annegers JH. (1962) **Glucose, amino acid and urea insorption from the canine intestine**. Proc Soc Exp Biol Med 111: 388-390. Glucose and glycine insorption rates increased in similar curvilinear fashion when 20 to 280 mM concentration of each was perfused through isolated jejunal or ilial loops in unanesthetlzed dogs. Lysine and histidine were insorbed at lower rates and urea was insorbed at a greater rate. Glycine and urea reduced glucose insorption rates when each was substituted for NaCl in the perfusion fluid, and glucose similarly reduced glycine insorption. ABSTRACT AUTHORS: John Annegers

Anonymous. (1991) **Urea kinetic modeling (UKM)**. Am J Kidney Dis 17: 244-245.

Bankowska J; Bojanowska A. (1973) **[Distribution of the activity of demethylases in some fractions of rat liver cells exposed previously to urea herbicides]**. Rocz Panstw Zakl Hig 24: 93-100.

Burg MB; Peters EM; Bohren KM; et al. (1999) **Factors affecting counteraction by methylamines of urea effects on aldose reductase**. Proc Natl Acad Sci USA 96: 6517-6522. The concentration of urea in renal medullary cells is high enough to affect enzymes seriously by reducing Vmax or raising Km, yet the cells survive and function. The usual explanation is that the methylamines found in the renal medulla, namely glycerophosphocholine and betaine, have actions opposite to those of urea and thus counteract its effects. However, urea and methylamines have the similar (not counteracting) effects of reducing both the Km and Vmax of aldose reductase (EC 1.1.1.21), an enzyme whose function is important in renal medullas. Therefore, we examined factors that might determine whether counteraction occurs, namely different combinations of assay conditions (pH and salt concentration), methylamines (glycerophosphocholine, betaine, and trimethylamine N-oxide), substrates (DL-glyceraldehyde and D-xylose), and a mutation in recombinant aldose reductase protein (C298A). We find that Vmax of both wild-type and C298A mutant generally is reduced by urea and/or the methylamines. However, the effects on Km are much more complex, varying widely with the combination of conditions. At one extreme, we find a reduction of Km of wild-type enzyme by urea and/or methylamines that is partially additive, whereas at the other extreme we find that urea raises Km for D-xylose of the C298A mutant, betaine lowers the Km, and the two counteract in a classical fashion so that at a 2:1 molar ratio of betaine to urea there is no net effect. We conclude that counteraction of urea effects on enzymes by methylamines can depend on ion concentration, pH, the specific methylamine and substrate, and identity of even a single amino acid in the enzyme.

Crosterveld MJS; Genke RJBJ; Dainty JR; et al. (2004) **In vivo measurement of urea production and recycling with [N-13(2)] urea: Underestimation of urea recycling rate by current methods**. Pediatr Res 55:187A.

Forsythe SJ; Parker DS. (1985) **Ammonia-nitrogen turnover in the rabbit caecum and exchange with plasma urea-N**. Br J Nutr 54: 285-292. Continuous infusion and single-shot administration of 15NH4Cl into the caecum of the conscious rabbit was used to measure caecal ammonia flux. Continuous infusion of 15NH4Cl and sampling from both the caecal ammonia and blood urea pools indicated that 0.27 of

plasma urea -nitrogen was derived from caecal ammonia-N. Values from intravenous [15N]urea and intracaecal 15NH4Cl infusions were used to produce two models of the movement of N between these two metabolic pools. Further analysis of the results suggested an alternative model involving a third pool associated with the caecal mucosa and values for this model are also presented.

Gobbato F. (1950) **[Relation between urinary volume and urea clearance; correction factor for clearance at reduced volume]. RAPPORTI TRA VOLUME URINARIO ED EPURAZIONE UREICA; FATTORE DI CORREZIONE PER LA CLEARANCE A VOLUME RIDOTTO**. Acta Med Patav 11: 37-42.

Holmes BE; Watchorn E. (1927) **Studies in the metabolism of tissues growing in vitro I. Ammonia and urea production by kidney**. Biochem J 21: 327-334. A study of the metabolism of growing mammalian tissues, through changes in NH3 and urea content of the embryo rat kidney. Growing rat kidney tissue produces considerable amounts of NH3 and urea; resting embryonic tissue produces neither. Brain tissue gave similar results. ABSTRACT AUTHORS: J. H. Barach

Iwamoto, K; Watanabe, J; Imai, K; et al. (1982) **Disposition of urea following intravenous administration to rats**. Chem Pharm Bull 30:1422-1429.

Keynes WM. (1971) **Equilibration of hypertonic solutions of urea and ammonia in the body fluids: study by following levels in blood and thoracic-duct lymph**. Ann Surg 174: 167-176.

Kim JR; Muresan A; Lee KYC; et al. (2004) **Urea modulation of beta-amyloid fibril growth: experimental studies and kinetic models**. Protein Sci 13: 2888-2898. Aggregation of beta-amyloid (Abeta) into fibrillar deposits is widely believed to initiate a cascade of adverse biological responses associated with Alzheimer's disease. Although it was once assumed that the mature fibril was the toxic form of Abeta, recent evidence supports the hypothesis that Abeta oligomers, intermediates in the fibrillogenic pathway, are the dominant toxic species. In this work we used urea to reduce the driving force for Abeta aggregation, in an effort to isolate stable intermediate species. The effect of urea on secondary structure, size distribution, aggregation kinetics, and aggregate morphology was examined. With increasing urea concentration, beta-sheet content and the fraction of aggregated peptide decreased, the average size of aggregates was reduced, and the morphology of aggregates changed from linear to a globular/linear mixture and then to globular. The data were analyzed using a previously published model of Abeta aggregation kinetics. The model and data were consistent with the hypothesis that the globular aggregates were intermediates in the amyloidogenesis pathway rather than alternatively aggregated species. Increasing the urea concentration from 0.4 M to 2 M decreased the rate of filament initiation the most; between 2 M and 4 M urea the largest change was in partitioning between the nonamyloid and amyloid pathways, and between 4 M and 6 M urea, the most significant change was a reduction in the rate of filament elongation.

Kist-van Holthe tot Echten JE; Nauta J; Hop WC; et al. (1992) **Protein intake can not be estimated from urinary urea excretion**. Pediatr Nephrol 6: 85-87. We assessed the relationship between protein intake (calculated from a 3-day prospective dietary diary) and 24-h urinary urea excretion in 37 children with chronic renal failure. Protein intake was not restricted during the investigation period. The 24-h urinary urea excretion correlated poorly with the protein intake estimated from the dietary diary (r = 0.58). We conclude that although it is common practice to assess compliance with a protein-restricted diet in children with chronic renal failure with a dietary diary and 24-h urinary urea excretion, the value of this assessment is questionable.

Kleinman LI; Radford EP; Torelli G. (1964) **[Clearance of urea and inulin in non-anesthetized rats not disturbed by technical handling]. EPURAZIONE DELL'UREA E DELL'INULINA IN RATTI NON ANESTETIZZATI E NON DISTURBATI DALLE MANUALITA TECNICHE**. Boll Soc Ital Biol Sper 40: 2235-2237.

Langeron L; Paget M; Liefooghe J. (1955) **[Mechanism of renal elimination of urea; application to urea clearance and understanding of hyperazotemia]. LE MECANISME DE L'ELIMINATION RENALE DE L'UREE; APPLICATION A LA CLEARANCE DE L'UREE ET A LA COMPREHENSION DES HYPERAZOTEMIES**. J Sci Med Lille 73: 8-9.

Miggiano GAD; Mordente A; Pischiutta MG; et al. (1986) **The kinetics of concurrent instantaneous and time-dependent inhibition of alkaline phosphatase by urea and related compounds**. Ital J Biochem 35: 33-41. Urea, thiourea and guanidine-HCl greatly inhibit bone Alkaline Phosphatase activity. The inhibition displays saturation kinetics, fitting a model for the reaction sequence in which formation of complexes with increasing affinity occurs. The time dependent inactivation by urea and guanidine follows pseudo-first order kinetics, while thiourea behavior is better described by a biexponential equation. The pH variation strongly affects the activity, the instantaneous inhibition and the time dependent inactivation. At the pH optimum the three compounds display their strongest effect. Data analysis suggests a three-stage model for the kinetics of Alkaline Phosphatase inhibition by urea and related compounds, involving a consecutive binding process with several sites of the protein and the production of different and interchanging inhibitor-enzyme complexes, leading to irreversibly inactivated forms.

Nomura, N; Matsumoto, S; Nishimura, Y; et al. (2006) **Disposition of exogenous urea and effects of diet in rats**. Arzneimittelforschung 56: 258-266. Although breath test using 13C-labeled urea (CAS 57-13-6, UBT) is becoming popular for the diagnosis of Helicobacter pylori (H. pylori) infection, disposition of exogenously given urea is not fully understood. The purpose of the present study is to elucidate the disposition of exogenous urea and to consider its relation with the UBT safety and biobehavior of endogenous urea. With 14C-labeled urea ([14C]urea), the absorption, distribution, metabolism and excretion including that into breathed air after its administration in trace to large doses in rats were investigated. [14C]Urea was given to fasted and non-fasted rats through intravenous and oral routes. It was found that the disposition of exogenous [14C]urea behaves in a similar way as endogenous urea, and a sufficiently large capacity for disposing urea in rats was suggested from the linear pharmacokinetics within the wide dose range of [14C]urea (2-1000 mg/kg). The safety of urea in UBT was also revealed by consideration of its dose and human urea body pool. It was also suggested that diet stimulates both systemic (as observed after the intravenous dose) and pre-systemic (as with the oral route) decompositions of urea into carbon dioxide and ammonia, but does not affect the renal elimination and distribution pattern in rat tissues. The findings in this study provide us with the quantitative information concerning not only the safety and disposition of urea as a diagnostic agent, but also the biobehavior of endogenous urea in ureotelism.

Oyanagi K; Nakamura K; Sogawa H; et al. (1980) **A study of urea-synthesizing enzymes in prenatal and postnatal human liver**. Pediatr Res 14: 236-241. The urea-synthesizing enzymes of human liver tissues, namely, carbamylphosphate synthetase (CPS, EC 2.7.2.2), ornithine transcarbamylase (OTC, EC 2.1.3.3), arginine synthetase system, argininosuccinase (ASase, EC 4.3.2.1), and arginase (EC 3.5.3.1) were measured between pre- and postnatal periods. Specimens from 67 autopsied human livers obtained from fetuses, premature infants, newborn infants, infants, children, and adults were examined. The mean activities of the enzymes showed an increased pattern for OTC and arginase at fetal life, whereas those of CPS, arginine synthetase system, and ASase of fetal livers showed no significant difference in each stage.

Except for arginase, the other four enzyme activities were higher in the postnatal period than those in the fetal life. Arginase activities indicated maximal increase at a gestational age between 28 and 31 weeks and decreased in the postnatal life.

Pocsi I; Berki AC; Taylor SA; et al. (1993) **Urea inhibition of urinary N-acetyl-?-D-glucosaminidase is mixed-type and sensitive to changes of pH away from the optimum**. Clin Chem 39: 1918-1919.

Sagardia F; Green JW. (1969) **Kinetic effects of urea on the activation of aged glycogen phosphorylase a by adenosine 5'-phosphate**. Biochim Biophys Acta 185: 80-87.

Shue GM; Douglass CD; Firestone D; et al. (1968) **Acute physiological effects of feeding rats non-urea-adducting fatty acids (urea-filtrate)**. J Nutr 94: 171-177.

Sjoberg B; Pap S; Jarnberg SE; et al. (1991) **Kinetics of the urea-induced dissociation of human plasma alpha 2-macroglobulin as measured by small-angle neutron scattering**. Biochem J 278: 325-328. The kinetics of the urea-induced dissociation of human plasma alpha 2-macroglobulin into two half-molecular fragments was investigated at 21.0 degrees C by using small-angle neutron scattering. The relative change in molecular mass that occurs upon dissociation was monitored by recording the forward scattering of neutrons as a function of time. All these kinetic data can be explained by a reaction that is first-order with respect to the concentration of undissociated alpha 2-macroglobulin. The velocity constant is a function of urea concentration and it varies within wide limits. For instance, the half-life of the reaction at the lowest concentration of [2H]urea studied (2.70 M) is 328 h, whereas the same value at the highest concentration of [2H]urea (6.24 M) is only 8 min. Measurements were made both with [1H]urea in 1H2O and with [2H] urea in 99% 2H2O, and it was found that there is a pronounced kinetic isotope effect, i.e. the dissociation is 4 times faster in the 1H-containing medium as compared with the 2H-containing medium at the same molar concentration of urea. From the angular dependence of the neutron scattering it can be concluded that the dissociation is associated with a drastic change in structure. This is directly shown by the radius of gyration, which increases from about 7.4 nm immediately after the addition of urea up to about 9.4 nm when the protein is fully dissociated. A structural analysis shows that the scattering curve of urea-dissociated alpha 2-macroglobulin can best be explained by that of a Gaussian coil with a radius of gyration equal to 9.44 nm. These data indicate that the so-called non-covalent interaction of alpha 2-macroglobulin probably is more complicated than just a pure hydrophobic interaction. Finally, it is also shown that the dissociation is accompanied by a loss in trypsin-binding activity, which is directly related to the fraction of dissociated protein.

Van Slyke DD; McIntosh JF; Moller, E; et al. (1930) **Studies of urea excretion. VI. Comparison of the blood urea clearance with certain other measures of renal function**. J Clin Invest 8: 357-374. In patients with diminishing renal function the blood urea clearance shows evidence of the diminution sooner than does the blood creatinine content, the blood urea content considered without relation to the urea excretion, or the phenolsulfonephthalein excretion. The blood urea clearance usually falls below 50% of its normal value before any of the other 3 values shows any abnormality. Only after the blood urea clearance indicates less than 20% of normal renal function are all values for blood urea and creatinine content, and for phenolsulfonephthalein excretion, found outside the limits of normal variation. In cases with diminishing renal function the phenolsulfonephthalein excretion is likely to register entirely normal values for some weeks or months after the blood urea clearance indicates less than 50% of average normal renal function. In cases improving from acute nephritis with severe functional deficit, the phenolsulfonephthalein excretion sometimes shows a marked rise several weeks in advance of rise in the blood urea clearance. In bladder retention, phthalein excretion is likely to be very low in comparison with the blood urea clearance , or the ratio Urea concentration in urine/Urea concentration in blood, because

of the fact that only a fraction of the phthalein excreted into the bladder is likely to be voided in 2 hrs. For this reason, comparison of phthalein excretion and blood urea clearance can be of assistance in detecting retention. A considerable degree of correlation was noted between the development of anemia and fall in blood urea clearance. Cases of chronic nephritis with less than 12 volumes % of blood O capacity, or 65% of Haldane's normal hemoglobin content, all showed less than 40 % of normal renal function measured by the blood urea clearance. However, hemoglobin content is likely to fall much later in the disease than the blood urea clearance. Hemoglobin contents over 80% of normal are maintained by some uremic cases with blood urea clearances reduced to less than 10% of normal. A low blood hemoglobin content in nephritis is a grave prognostic sign, but a fairly good hemoglobin content does not indicate in every case a better prognosis. The simple concentration ratio, Urea concentration in urine/Urea concentration in blood, observed when the urine volume is less than 2 cc. per min., preferably less than 100 cc. per hr., has approximately the same mean normal numerical value, about 50, as the standard blood urea clearance, and is equally sensitive to diminution in renal function. In a given individual the ratio shows more variation than the clearance because the ratio lacks correction for urine volume changes; but it serves well as a substitute for the clearance when, because of incontinence, retention, or other reason, the urine volume output within a definite period can not be measured. ABSTRACT AUTHORS: Authors' summary

Wolfe RR; Evans JE; Durkot MJ; et al. (1980) **Isotopic studies of urea kinetics**. Fed Proc 39: 13-18.

Yang D; Hazey JW; David F; et al. (1997) **Kinetics and transorgan balance of ammonia (AM), glutamine (GLN) and urea in dogs, investigated by mass isotopomer distribution (MID) analysis**. FASEB J 11: 6-9.

3.1. ABSORPTION

Dawes, C. (2006) **Absorption of urea through the oral mucosa and estimation of the percentage of secreted whole saliva inadvertently swallowed during saliva collection**. Arch Oral Biol 51: 111-116. OBJECTIVE: To determine whether some of the urea added to certain chewing gums may be absorbed through the oral mucosa and whether some saliva is inadvertently swallowed during the collection of saliva elicited by the chewing of gum. DESIGN: On two occasions, 10 experienced saliva collectors made a 5 min collection of unstimulated whole saliva and then chewed gum for 10 min and during this time collected their saliva. On one occasion, they chewed one tablet of gum containing 0.5 mg of Phenol Red, a non-absorbable substance, and one tablet of a gum containing 27.3 mg of urea. On another occasion, they chewed two tablets of the Phenol Red gum. Their saliva and the chewed gum were assayed for their Phenol Red and urea contents and the totals calculated. Since saliva normally contains urea, the recovery of urea was calculated as the difference between the amounts recovered in the two collection sessions. RESULTS: The mean recovery of Phenol Red was 96.7%, but in three participants the amount recovered was less than the 95% confidence limits for assay error. The mean recovery of urea was 85.7% and in nine of the 10 participants, the amount recovered was less than the confidence limits for assay error. In all participants, the percentage urea recovery was less than that of Phenol Red. CONCLUSION: The results showed: (1) that Phenol Red appears to be a useful, non-absorbed marker for studies of drug absorption through the oral mucosa, (2) that when the salivary urea concentration is higher than that in plasma, urea may be absorbed through the oral mucosa, (3) that even experienced saliva collectors may inadvertently swallow some of the saliva they produce. This latter finding has implications for all clinical studies of saliva.

Enna, SJ; Schanker, LS. (1972) **Absorption of saccharides and urea from the rat lung**. American J Physiol 222: 409-414.

Mariotti, F; Pueyo, ME; Tome, D; et al. (2001) **Guar gum does not impair the absorption and utilization of dietary nitrogen but affects early endogenous urea kinetics in humans**. Am J Clin Nutr 74: 487-493. BACKGROUND: Viscous gums enhance viscosity in the upper gastrointestinal lumen, quickly disturbing motility and promoting fluid secretion. OBJECTIVE: We sought to determine whether guar gum could acutely affect the absorption and utilization of dietary nitrogen and whether these luminal effects could also perturb the kinetics of urea. DESIGN: We studied the short-term effect of adding 1% of highly viscous guar gum to a (15)N-labeled protein meal (30 g soy protein isolate in 500 mL water) during the postprandial phase in humans. The effects on bioavailability were studied by using the [(13)C]glycine breath test (to assess gastric emptying) and (15)N enrichment in plasma amino acids (for systemic amino acid bioavailability). The kinetics of dietary and endogenous urea were assessed in plasma and urine. RESULTS: Guar gum modulated the gastric emptying kinetics of the liquid phase of the meal slightly ($P < 0.05$), but had no significant effect on either the systemic appearance of dietary amino acids or plasma and urinary dietary urea kinetics. Without significantly affecting plasma urea concentrations, guar gum reduced by approximately 40% the urinary excretion of endogenous urea for the first 2-h period after the meal ($P < 0.01$), although endogenous urinary excretion was similar at later stages. CONCLUSIONS: Guar gum did not significantly affect the bioavailability or utilization of dietary protein. We showed an early effect of guar gum on endogenous urea kinetics, which most probably arose from very early, short-term stimulation of the intestinal disposal of endogenous urea, at the expense of its urinary excretion.

Ochsenfahrt, H; Winne, D. (1973) **The contribution of solvent drag to the intestinal absorption of tritiated water and urea from the jejunum of the rat**. Naunyn Schmiedebergs Arch Pharmacol 279: 133-152.

3.2. DISTRIBUTION

Audi, SH; Dawson, CA; Linehan, JH; et al. (1996) **An interpretation of 14C-urea and 14C-primidone extraction in isolated rabbit lungs**. Ann Biomed Eng 24: 337-351. We measured the venous concentration versus time curves of 14C- urea and 14C-primidone after rapid bolus injections of a vascular reference indicator, fluorescein isothiocyanate dextran, and one of the two 14C- labeled indicators in isolated rabbit lungs perfused with Krebs-Ringer bicarbonate solution containing 4.5% bovine serum albumin at flow rates (F) of 6.67, 3.33, 1.67, and 0.83 ml/sec and with nearly constant microvascular pressure and total lung vascular volume. When we calculated the permeability- surface area product, PS, from the 14C-urea and 14C-primidone outflow curves using the Crone model, the estimates of the PS product were directly proportional to F. However, the fractional change in the PS with flow was different for the two indicators. We also estimated the PS from the same 14C- urea and 14C-primidone data using an alternative model that includes perfusion heterogeneity, estimated in a previous study, and flow-limited and barrier-limited extravascular volumes accessible to both urea and primidone. This model was able to fit the outflow curves of either 14C-urea or 14C- primidone at all four flows studied with one flow-independent PS for each indicator. The ability of the new model to explain the 14C-urea and 14C- primidone data with no flow-dependent change in PS suggests that a change in PS with F estimated using other models such as the Crone model is not sufficient evidence for capillary surface area recruitment.

Boccl, V. (1963) **The measurement of the volume of total distribution of urea in the body during the use of urea C14. Original Title: SULLA MISURAZIONE DEL VOLUME DI DISTRIBUZIONE TOTALE DELL' UREA NEL CORPO MEDIANTE UREA C14**. Atti Accad Fisiocrit Siena Sez Med

Fis 12: 1127-1130. The distribution of urea-C14 in the rabbit was determined. The dosage lost in the urine immediately after intravenous injection of label was found to be 10%. Urea-Cl4 was detected in the urine 4 to 10 min. after injection and the radioactivity decreases more slowly in urine than in plasma. ABSTRACT AUTHORS: G. Hanson

Dresser, TP; Nolph, KD; Vitale, FC. (1977) **Transerythrocytic concentrations of urea and creatinine in normals and in azotemic patients**. Int Urol Nephrol 9: 169-176. Preliminary studies in our laboratory suggested alterations in the distribution of urea and creatinine between red cells and plasma in azotemic patients, as compared to normal controls. The present studies compare plasma erythrocyte concentrations of urea and creatinine in azotemic patients and in control subjects. Effects of adding exogenous urea and creatinine to the blood of control subjects were also studied. The findings suggest alterations of solute distribution patterns in azotemic blood. Specifically, the proportions of the solutes in cells appear reduced in azotemia.

Johanson, CE; Woodbury, DM. (1978) **Uptake of [14C]urea by the in vivo choroid plexus--cerebrospinal fluid--brain system: identification of sites of molecular sieving**. J Physiol 275: 167-176. 1. The time course of the uptake of [(14)C]urea by the lateral ventricular choroid plexus of the adult rat in vivo was analysed to delineate further the permeability characteristics of the epithelial membrane of this secretory tissue.2. Eight hours after I.P. injection, [(14)C]urea attained a steady-state distribution in 78% of the tissue water of lateral ventricular choroid plexus; similarly, approximately 8 hr was required for radiourea to reach a steady-state concentration in both the cerebral cortex and cerebrospinal fluid (c.s.f.).3. Results obtained for compartment analysis were used to calculate the concentration of [(14)C]urea in the epithelium of the lateral ventricular plexus during the approach to and at steady-state distribution. Even after 1 hr of distribution, the [(14)C]urea concentration in choroid cell water was less than 15% of that in plasma water.4. Although the concentration of radiourea in choroid cell water continually increased after 3 hr, it remained in equilibrium with the concentration of [(14)C]urea in c.s.f. water. At the steady state (i.e., 8 hr), the distribution of [(14)C]urea between the water of plasma and that of the choroidal epithelium was considerably away from equilibrium (i.e., by 25-30%).5. An analysis of the concentration gradients for [(14)C]urea across both the apical (c.s.f.-facing) and basolateral (plasma-facing) membranes of the epithelium of the lateral ventricular plexus suggests that the movement of urea is hindered to a greater extent by the basolateral membrane than by the apical membrane.6. Only a single half-time component (1.3 hr) can be resolved from analysis of the curve describing the time course of uptake of radiourea by the choroid epithelial cell compartment.7. The concentration gradient data suggest that urea penetrates from blood to c.s.f. via the choroid plexus by a transcellular pathway; however, it is not possible to rule out a paracellular pathway for urea movement.8. At the steady state, radiourea distributes into 88% of the water of cerebral cortex. This observation, together with the finding of a steady-state concentration gradient for [(14)C]urea from cortical tissue to c.s.f., constitutes evidence that urea movement is hindered at the blood-brain barrier as well as at the blood-c.s.f. barrier.

Martinez, DG; Auchus, R; Saad, TF. (1993) **Determining urea distribution volume by dual-energy X-ray absorptiometry (DEXA)**. J Am Soc Nephrol 4: 14-17.

Okumura, K; Lee, IP; Dixon, RL. (1975) **Permeability of selected drugs and chemicals across the blood-testis barrier of the rat**. J Pharmacol Exp Ther 194: 89-95. Physiological studies by Setchell and others have described the existence of a blood-testis barrier (BTB) surrounding the seminiferous tubules of the mammalian testis. These studies were initiated to better define the role of the BTB with regard to the penetration of exogenous chemicals to male germ cells. The rete testis was cannulated in rats and fluid was collected. Test chemicals or drugs were usually administered by continuous i.v. infusion. Permeability of nonelectrolytes of various molecular sizes, acidic compounds with varying partition

coefficients and pKalpha values, such as salicylic acid, barbiturates, and sulfonamides, across the BTB were studied. Permeability of nonelectrolytes was demonstrated to be dependent upon their molecular size, suggesting bulk flow through water-filled pores. On the other hand, permeability of acidic drugs with varying pKalpha values depended upon their partition coefficients. Transport of these chemicals from blood to seminferous tubules closely resembled their transport from blood to cerebrospinal fluid. It appears that the BTB is a complex multicellular system composed of membranes surrounding the semiferous tubules and the several layers of spermatogenic cells organized within the tubules, which restrict the permeability to the male germ cells of many foreign compounds. This must be borne in mind when extra-polating data from in vitro mutagenic test systems to man.

Rapoport, SI; Fitzhugh, R; Pettigrew, KD; et al. (1982) **Drug entry into and distribution within brain and cerebrospinal fluid: [14C]urea pharmacokinetics**. Am J Physiol 242: R339-348. A four-compartment model was derived to analyze drug exchange among cerebral capillary plasma, cerebrospinal fluid (CSF), and the brain extracellular and intracellular (or bound) compartments. Equations that were derived incorporated the factor of cerebral blood flow. They were fit by nonlinear least squares to measured brain, plasma, and CSF (when available) concentrations of [14C]urea in the rat, in response to a step increase in plasma concentration, to intravenous infusion, or to a bolus injection of tracer. Best-fit values for the transfer constants were consistent among the three administrative regimens and agreed with published values, when available. Expressions also were derived and numerically evaluated for the lower limit of the brain extracellular space, for half times of brain [14C]urea uptake, and for the steady-state brain/plasma distribution volume. The model should make it possible to use transfer constants obtained for a given drug from one study (e.g., constant plasma concentration) to predict brain concentrations from measured plasma concentrations in other acute or chronic studies.

Sahin, S; Rowland, M. (2007) **Influence of erythrocytes on the hepatic distribution kinetics of urea and thiourea**. Eur J Pharm Sci 31: 180-189. The role of erythrocyte on the hepatic distribution kinetics of urea and thiourea was investigated in the in situ isolated perfused rat liver. Perfusion experiments were conducted using Krebs-bicarbonate buffer delivered via the portal vein in a single pass mode at a total flow rate of 15ml/min. With urea, superimposable unimodal effluent curves were obtained in the presence and absence of erythrocytes, indicating that its distribution kinetics is not affected by erythrocytes. With thiourea, effluent curves were unimodal in the absence of erythrocytes but bimodal in the presence of erythrocytes. The maximum frequency output at the first peak increased from 0.017+/-0.002 to 0.042+/-0.006s(-1) with an increase in the bolus hematocrit from 0.40 to 0.75, indicating that some thiourea fraction is retained by the erythrocytes on transit through the liver. Although the fractional output associated with the first peak was very similar (11.9% versus 11.5%), whether the perfusate contained unlabelled thiourea or not, this fraction was reduced from 17 to 5% with a decrease in the incubation time before injection from 30min to 40s. However, there was no evidence for a capacity limitation; a 30-min period of pre-incubation of either radiolabelled thiourea alone or combined with a high concentration of unlabelled thiourea had minimal effect on effluent profiles.

Simenhoff, ML; Dunn, S; Hilton, B. (1972) **Role of Regional Brain Urea Distribution in Uremic Encephalopathy**. Clin Res 20: 609.

Waddell, WJ. (1968) **Distribution of urea-14C in pregnant mice studied by whole-body autoradiography**. J Appl Physiol 24: 828-831.

3.2.1 Transfer/Distribution in Mother and Fetus

Abramovich, DR; Heaton, B; Page, KR. (1974) **Transfer of labelled urea, creatinine and electrolytes between liquor amnii and the fetoplacental unit in midpregnancy**. Eur J Obstet Gynecol Reprod Biol 4: 143-146.

Battaglia, FC; Bruns, PD; Behrman, RE; et al. (1964) **Comparsion of Permeability of Different Layers of the Primate Placenta to D-Arabinose and Urea**. Am J Physiology 207: 500-502.

Bissonnette, JM; Cronan, JZ; Richards, LL; et al. (1979) **Placental transfer of water and nonelectrolytes during a single circulatory passage**. Am J Physiol 236: C47-52. A bolus of a 14C-labeled nonelectrolyte and tritiated water was injected into the maternal arterial system supplying the uterine circulation. Blood was sampled immediately on the fetal side of the placenta from the umbilical vein. A placental transfer index (PTI) was calculated from the ratio of the tracers in the fetal blood divided by the ratio of the tracers in the injectate. Placental transfer was measured for 11 nonelectrolytes in anesthetized guinea pigs and 8 nonelectrolytes in unanesthetized sheep. Placental transfer in both species was determined by molecular weight and lipid solubility. The PTI measured for the lipophilic solutes antipyrine and 1,6-hexanediol were less than 1, and those for methanol and ethanol were greater than 1. We conclude that placental transfer for water and small (mol wt less than 200) lipid-soluble nonelectrolytes is limited by diffusion as well as by blood flow.

Blake, DA; Burnett, LS; Miyasaki, BC; et al. (1976) **Pharmacokinetics of intra-amniotically administered hyperosmolar urea in rhesus monkeys**. Am J Obstet Gynecol 124: 245-250. Three rhesus monkeys in midgestation were injected intra-amniotically with hyperosmolar 14C-urea (58 per cent weight/volume, 2.3 Gm. per kilogram, 25 muc) and the concentration of 14C-urea measured serially for 24 hours in amniotic fluid, maternal serum, and maternal urine. The volume of the amniotic space was measured by isotope dilution of 99mTc-albumin. The experimental values were fitted to a three-compartment mathematical model with the aid of a digital computer program for nonlinear data. The computer-derived concentration versus time curves were in close agreement with the measured values when the flux of urea was primarily from amniotic fluid to fetal water to maternal water with insignificant direct transport between the amniotic fluid and maternal serum. The half-times for distribution of urea into fetus and mother were approximately 30 minutes and 7 hours, respectively. Thus, although the uptake of urea by the fetus from surrounding amniotic fluid is rapid, the further movement of urea from fetus to mother is relatively slow.

Dancis, J; Kammerman, S; Jansen, V; et al. (1981) **Transfer of urea, sodium, and chloride across the perfused human placenta**. Am J Obstet Gynecol 141: 677-681. Clearance indices (clearance of experimental material: clearance of antipyrine, CI) across the perfused human placenta were determined for urea, sodium, and chloride. The selected materials are of relatively small molecular weight and are water soluble. Clearance of urea was determined under conditions of net transfer and of exchange and no difference was detected. Sodium and chloride clearances were measured as exchange rates. The CI were: urea, 0.32 and 0.38 toward fetus and mother, respectively); sodium, 0.28; and chloride, 0.41 (measured toward the fetus). Recalculation of data as the diffusion limitation (LD) facilitated comparison with data in the literature on the monkey, rabbit, and sheep. LD for urea was comparable among the four species whereas the sheep placenta differed, sharply limiting the diffusion of sodium and chloride. The discrepancy in the sheep between urea and sodium indicates that the low transfer rate of the latter cannot be explained by the "thickness" of the syndesmochorial placenta, as is often stated. Inferences concerning nitrogen metabolism in the fetus have been derived by others from data on transplacental urea gradients

and urea clearance. Available data in the human are incomplete but are consistent with amino acids providing approximately 10% of fetal energy needs, with glucose serving as the predominant substrate.

Faber, JJ; Hart, FM (1967) **Transfer of charged and uncharged molecules in the placenta of the rabbit**. Am J Physiol 213: 890-894.

Gresham, EL; Simons, PS; Battaglia FC. (1971) **Maternal-fetal urea concentration difference in man: metabolic significance**. J Pediatr 79: 809-811.

Grynfogel, M; Hutchinson, DL; Kelly, WT; et al. (1962) **[Placental transmission of C-14-labeled urea in primates.]**. C R Hebd Seances Acad Sci 254: 2444-2446.

Hisanaga, H; Iioka, H; Moriyama, I; et al. (1991) **The mechanism of human placental urea transport: a study using placental brush border (microvillous) membrane vesicles**. Asia Oceania J Obstet Gynaecol 17: 67-72. To elucidate the mechanism of placental amino acid transport, the transport of amino acids into brush border membrane vesicles was investigated using brush border membrane vesicles (BBMV) separated from the human full-term placenta. 1. The transport of l-alanine and l-glutamine into the brush border membrane vesicles prepared from either early gestational placenta or full-termed placenta disclosed a typical overshooting phenomenon in the presence of H+ concentration gradient (extravesicular pH greater than intravesicular pH). 2. The H(+)-dependent overshooting transport of urea into the brush border membrane vesicles disappeared in the presence of H+ ionophore. 3. The initial velocity of H+ concentration gradient dependent uptake of urea into the brush border membrane vesicles was regulated by the saturation kinetics determined by the concentration of urea. The values of Km and Vmax, calculated as the kinetic parameters of urea transport by reciprocal plotting of the initial velocity of uptake and the concentration of urea, were 10.8 mM and 410 mumol/mg protein/10 sec, respectively. The above results indicate that a transport system which transports urea in exchange for H+ exists in human full-term placental microvillous membrane.

Horn, J. (1978) **Excretion of urea by the foetal guinea pig**. Clin Sci Mol Med 55: 329-333. 1. The placental transfer of urea was studied by perfusing the guinea-pig foetal placenta in situ with dextran solutions containing various amounts of urea, and radioactively labelled urea. 2. Transfer of urea was linearly related to the difference in concentration between the maternal and the foetal sides of the placenta, but transfer in both directions across the placenta was equal when the concentration of urea in the perfusing fluid was 2.5--3.5 mmol/l less than the maternal arterial value. This suggested that urea may be transferred against a concentration gradient. 3. Foetal plasma urea concentrations were found to be 0.5 mmol/l less than the maternal, suggesting that active transfer from the foetal circulation to the maternal can occur. However, because of the close relationship between foetal and maternal plasma urea (r = 0.96), it is concluded that the major control of foetal urea concentrations is by diffusion of urea between maternal and foetal extracellular fluids.

Hutchinson, DL; Kelly, WT; Friedman, EA; et al. (1962) **The distribution and metabolism of carbon-labeled urea in pregnant primates**. J Clin Invest 41: 1745-1753. The synthesis of urea-C-13 from NaCN and its analysis in body fluids is described. Pregnant rhesus monkeys at term were subjected to routine operative procedures for the placement of catheters into maternal arteries, into placental vessels and amniotic fluid. A mixture of carbon-14 urea and tritium oxide was injected into one of these compartments and samples collected over a period of several hours. In two additional experiments urea-C-13 and C-14 were injected simultaneously into fetal and maternal bloodstreams. The specific activity curves for the first set of experiments indicated that radiourea is distributed in a general urea pool, that there is a rapid exchange of urea between maternal and fetal blood and an appreciably slower transfer to

the amniotic fluid whose urea is derived from mother and fetus in an approximate ratio of 31. The exchange rate between maternal and fetal blood could be estimated as 1 to 2 millimoles of urea/kilogram fetus/hour. The maximal rate of endogenous urea production in the fetal organism amounts to less than 1/10 of the total placental exchange. ABSTRACT AUTHORS: Authors

Luck, JM; Engle, ET. (1929) **The permeability of the placenta of the rat to glycine, alanine and urea**. Am J Physiol 88: 230-236. Glycine, alanine and urea were administered by subcutaneous injection to pregnant rats on the 18th or 19th day of gestation. An amount equal to 0.4 gm. N per kgm. of body weight was given in 5 cc. of 0.9% NaCl after a 20 hr. fast. With glycine and alanine, the amino N content of the whole fetus and of the maternal muscle and liver rose considerably, at similar rates, indicating that the placenta is freely permeable to amino acids. The mechanism by which the maternal muscle absorbs amino acids from the maternal plasma is the same as that by which the fetus absorbs amino acids from the fetal plasma; namely, diffusion. The fact that at birth the fetal plasma has a higher concentration of amino acids than the maternal plasma may be due to its lower pH value and consequent influence on the distribution across the placenta] membrane. Injection of urea led to a large increase in urea in the fetus and in the maternal muscle and liver. ABSTRACT AUTHORS: D. J. Lloyd

Moore, WM; Ward, BS. (1970) **Placental membrane permeability to creatinine and urea**. Am J Obstet Gynecol 108: 635-637.

Moore, WM. (1971) **Placental permeability to creatinine and urea**. J Reprod Fertil 25: 456.

Roller, MH; Swanson, RN; Lang ML. (1970) **Blood and tissue ammonium nitrogen and tissue water values in urea -treated pregnant rabbits**. Am J Vet Res 31: 327-330.

Stanier, MW. (1965) **Transfer of radioactive water, urea and glycine between maternal and foetal body fluids in rabbits and pigs**. J Physiol 178: 127-140.

Thornburg, KL; Faber, JJ. (1977) **Transfer of hydrophilic molecules by placenta and yolk sac of the guinea pig**. Am J Physiol 233: C111-124. Prenatal transfer of hydrophilic materials was studied in chronic guinea pig preparations of ca. 30-65 days gestation. In most preparations the vitelline (yolk sac placenta) circulation of one of the fetuses was ligated between 1 and 20 days before the experiment. Fetal and maternal serum protein concentrations were recorded as a function of the number of days after ligation. Prenatal transfer of hydrophilic materials of 60-5,500 daltons molecular wt was consistent with diffusion, with some evidence of steric restriction of the larger molecules. There was no difference between operated and sham-operated or control fetuses. However, the transfer of radiolabeled homologous gamma globulin ceased after ligation of the yolk sac vessels. The transfer in control fetuses accounted for most of the prenatal requirements of this protein, as calculated from the increases in fetal weight and serum gamma globulin concentrations as a function of gestational age. There was evidence of a nonspecific suppression of yolk sac function by surgery but no evidence of fetal catabolism of gamma globulin.

Waddell, WJ. (1968) **Distribution of urea-14C in pregnant mice studied by whole-body autoradiography**. J Appl Physiol 24: 828-831.

3.3. METABOLISM

Anonymous. (1981) **Short-term regulation of urea synthesis**. Nutr Rev 39: 219-221.

Bean ES; Atkinson DE. (1984) **Regulation of the rate of urea synthesis in liver by extracellular pH. A major factor in pH homeostasis in mammals**. J Biol Chem 259: 1552-1559. The views that catabolism of protein leads to net production of acid and that urinary excretion of ammonium ion represents an equimolar excretion of proton are not compatible with basic chemical relationships (Atkinson, D.E., and Camien, M.N. (1982) Curr. Top. Cell. Regul. 21, 261-302). Metabolism of protein produces significant amounts of base (bicarbonate), which is disposed of in the synthesis of urea. In perfused rat liver and in isolated rat hepatocytes, the rate of urea synthesis increases with increase in pH but is not affected by change in the concentration of bicarbonate when pH is held constant. An increase in the concentration of ammonium ion in the suspending medium causes an increase in the rate of urea synthesis by hepatocytes when lactate is the energy source, as previously reported by others, but causes a decrease in the rate of urea synthesis during incubation with glucose or with no added energy source. The rate of urea synthesis decreases when glucose is added to lactate medium. All of these observations are consistent with the view that disposal of bicarbonate is a major function of urea synthesis, and that regulation of the rate of ureagenesis is an important factor in the maintenance of pH homeostasis.

Berezov TT. (1959) **Current views on the process of urea formation in animals**. Clin Chem 5: 218-238. A review with 93 references (33 from Russian literature) concerned with the route by which the final products of N metabolism are formed in mammals and man. Urea formation mechanisms in animals are reviewed in the light of current knowledge and information of specific enzyme reactions, oxidative deamination and reductive amination. The author cites his work and that of Russian researchers which shows that NH3 is the direct source of one urea N atom introduced in the synthesis of citrulline from ornithine, while the other N atom is derived from L-aspartic acid in the transformation of citrulline into arginine. The main routes of N assimilation by mammals show that N from all the amino acids and from other N compounds that finally end up as urea must either be utilized as NH3, directly or by reamination, or be transformed to NH2 in L-aspartic acid. ABSTRACT AUTHORS: Author

Brewer TG; Berry WR; Harmon JW; et al. (1984) **Urea synthesis after protein feeding reflects hepatic mass in rats**. Hepatology 4: 905-911. Urea synthesis is an exclusive biosynthetic function of the liver. Since the exact relationship between urea synthesis in vivo and functional liver mass remains unclear, we established an animal model using oral protein loading and measurement of resultant urea synthesis in rats. We studied rats subjected to sham operation, 40% hepatectomy, 66% hepatectomy, portacaval shunt and CCl4-induced cirrhosis. Urea synthesis was calculated as the sum of urinary urea excretion and accumulation of urea in body water during the 6-hr period after oral administration of a casein protein load equivalent to 20 gm per kg body weight. Peak urea synthesis rate in the sham-operated group of rats was 142 +/- 11 mumoles per hr per gm wet liver weight (mean +/- 1 S.D.), 473 +/- 34 mumoles per hr per gm liver protein and 80 +/- 5 mumoles per hr per mg liver DNA. This rate closely matched those of the other groups for each type of liver mass measurement. Marked reduction (p less than 0.01) of urea synthesis on a DNA basis was noted only in the CCl4-cirrhotic livers, related to the significantly higher (p less than 0.01) DNA content of the cirrhotic livers. Similarly, increased (p less than 0.05) liver protein content of the sham-operated rats when compared with the other groups was reflected in slightly lower urea synthesis rates expressed on the basis of liver protein (p less than 0.05) when compared to that of all other groups.(ABSTRACT TRUNCATED AT 250 WORDS)

Brosnan JT; Brosnan ME; Charron R; et al. (1996) **A mass isotopomer study of urea and glutamine synthesis from 15N-labeled ammonia in the perfused rat liver**. J Biol Chem 271: 16199-16207. This

study examines the incorporation of 15N from 15NH4Cl into urea and glutamine, predicts the pattern of isotopomers produced as a function of the 15N enrichment of the relevant precursor pools, and presents a means of determining the isotopic enrichment of these pools. Rat livers were perfused, in the nonrecirculating mode, with 0.3 mM 15NH4Cl, and the isotopomers of urea and of glutamine produced were determined by gas chromatography-mass spectrometry methodology. Three different nitrogen mass isotopomers of urea were found, containing no, one, or two atoms of 15N. Four glutamine isotopomers were found, containing no 15N, one atom of 15N in either the amino or amide position, or two 15N atoms. A mathematical relationship was deduced that predicts that the relative proportions of the urea isotopomers depends not only on the relative enrichment of 15N in the two precursor pools of urea nitrogen (mitochondrial ammonia and cytoplasmic aspartate) but on their absolute enrichment. This relationship was validated in experiments in which the isotopic enrichment of the substrate, 15NH4Cl, was varied. The proportions of the urea isotopomers produced can be predicted if one knows the 15N enrichment in the two precursor pools. We found that when the 15N enrichment of citrulline and aspartate in the perfusate were used as proxies for that in the mitochondrial ammonia and cytoplasmic aspartate pools we could accurately predict the relative proportion of the three isotopomers. The production of the four nitrogen isotopomers of glutamine could be used to determine the 15N enrichment in the two precursor pools of glutamine nitrogen, the cytoplasmic ammonia and glutamate pools of the perivenous hepatocytes.

Canioni P; Sciaky M; Masson S; et al. (1989) **Detoxification by glutamine and urea synthesis carbon-13 NMR study of the perfused rat liver**. Gastroenterol Clin Biol 13: 13-15.

Charbonneau R; Roberge A; Berlinguet L. (1967) **Variation with age of the enzymes of the urea cycle and aspartate transcarbamylase in rat liver**. Can J Biochem 45: 1427-1432.

du Toit, PJ; van Aswegen, CH; Nel, JA; et al. (1995) **In vivo effects of urease-producing bacteria involved with the pathogenesis of infection-induced urolithiasis on renal urokinase and sialidase activity**. Urol Res 23: 335-338. Many hypotheses have been proposed for renal stone formation. It has been argued that with infection-induced renal stones the hydrolysis of urea by bacterial urease increases urinary pH, with consequent stone formation. Unfortunately, this theory is not applicable to the micro-organisms that do not produce urease (e.g. Escherichia coli). It has been recently reported that E. coli reduces the urinary urokinase activity of male rats, but does not influence the urinary sialidase activity. This study has now been expanded to the urease-producing bacteria Proteus mirabilis, Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa and Micrococcus luteus. Subcutaneous injections with these bacteria were found to significantly ($P < 0.003$) reduce the UK activity of extrarenally obstructed kidneys. The urease-producing mammalian skin bacterium, M. luteus, was, however, the exception ($P = 0.1079$). In contrast to S. epidermidis, P. aeruginosa and M. luteus ($P < 0.0213$), P. mirabilis and S. aureus had no effect on renal sialidase activity ($P < 0.4047$). These results may explain why Proteus species are predominant in infection-induced renal stones. According to the urokinase-sialidase hypothesis, a decrease in urinary urokinase activity should increase the uromucoid levels, whilst no effect on the urinary sialidase activity should favour conversion of urinary uromucoid to mineralizable matrix. These conditions may lead to renal stone formation. An increase in urinary pH resulting from urease-producing micro-organisms will increase salt precipitation on the uromucoid. It is thus concluded that urease-producing bacteria may play a double role in renal stone formation.

Ejiri K. (1980) **Studies on entero hepatic circulation of urea nitrogen in pregnant rat (author's transl)**. Nippon Sanka Fujinka Gakkai Zasshi 32: 601-610. The purpose of this paper is to investigate the specific metabolism of the protein and amino acid during pregnancy from a standpoint of urea nitrogen recycling hydrolyzed by intestinal bacterial urease of pregnant rat. For this purpose, the activity of urease

of the intestinal flora, and L-glutamate dehydrogenase (GDH) in liver mitochondria, the concentration of free ammonia and urea in the intestinal tract, portal vein and right ventricle of the rat were discussed. The results were: 1) The activity of urease moderately increased during pregnancy with the peak on 19th gestational day. 2) The concentration of free ammonia in the intestinal tract elevated slightly, and markedly elevated in portal vein, but seemed to be no specific change in right ventricle. The peak showed on 19th gestational day. 3) The activity of GDH increased markedly during pregnancy, and the protein synthesis was thought to be accelerated. 4) Urea concentration in intestinal tract and blood stream seemed somewhat increased. This results revealed that the urea recycling system and protein synthesis accelerated during pregnancy because of high urease and GDH activity. This phenomenon adapted the pregnant to nutrient of the fetus for growing and development, and introduced a new concept of maternal-fetal unit of nutrition, especially in protein metabolism.

Felipo V; Minana MD; Grisolia S. (1991) **Control of urea synthesis and ammonia utilization in protein deprivation and refeeding**. Arch Biochem Biophys 285: 351-356. Rats were fed a standard diet (20% protein) or a protein-free diet for up to 65 days. After 20 days on the protein-free diet some rats were refed the standard diet. By the 20th day the rats fed the protein-free diet showed a blood ammonia level approximately 70% higher than controls and urea excretion decreased approximately 20-fold. At this time the liver acetylglutamate decreased to approximately one-fifth of the initial and control levels, returning to normal after 3 days of refeeding the standard diet, with a concomitant increase in urea excretion. The protein-deficient diet resulted in decreased activities of liver enzymes related to ammonia metabolism. All enzyme activities assayed returned to normal values rapidly upon refeeding the standard diet, except hepatic carbamylphosphate synthetase, glutamine synthetase, and glutaminase, which took approximately 1 month to return to control values. The findings presented here are consistent with the view that urea production is controlled, at least under certain conditions, by acetylglutamate, the physiological activator of carbamylphosphate synthetase.

Forsythe, SJ; Parker, DS. (1985) **Urea turnover and transfer to the digestive tract in the rabbit**. Br J Nutr 53: 183-190. 14C and 15N isotopes of urea were infused intravenously into rabbits for 6-8 h in order to measure urea synthesis and the extent of degradation in the digestive tract. The results indicate that 0.62 of the urea flux was excreted in the urine and that re-incorporation of urea-N following hydrolysis in the gut represented 0.3 of the urea synthesis rate. Sampling of metabolites from the caecum by dialysis provided an opportunity to assess the contribution of urea-N to the caecal ammonia pool. This contribution is calculated to be 0.25 of caecal ammonia turnover. Infusion of a urease (EC 3.5.1.5) inhibitor during a continuous infusion of [14C]urea into the caecum permitted the measurement of urea turnover within the caecum. Results obtained for urea entry into the caecum are contrasted with the measured urea degradation rate in the gut.

Forsythe, SJ; Parker, DS. (1985a) **Ammonia-nitrogen turnover in the rabbit caecum and exchange with plasma urea-N**. Br J Nutr 54: 285-292. Continuous infusion and single-shot administration of 15NH4Cl into the caecum of the conscious rabbit was used to measure caecal ammonia flux. Continuous infusion of 15NH4Cl and sampling from both the caecal ammonia and blood urea pools indicated that 0.27 of plasma urea -nitrogen was derived from caecal ammonia-N. Values from intravenous [15N]urea and intracaecal 15NH4Cl infusions were used to produce two models of the movement of N between these two metabolic pools. Further analysis of the results suggested an alternative model involving a third pool associated with the caecal mucosa and values for this model are also presented.

Gibson, JA; Sladen, GE; Dawson, AM. (1973) **Proceedings: Studies in the role of the colon in urea metabolism**. Gut 14: 816.

Gilboe, DD; Javid, MJ. (1964) **Breakdown products of urea and uremic syndrome**. Proc Soc Exp Biol Med 115: 633-637. The objective was to determine whether the reported toxicity of urea in nephrectomized dogs was due to ammonia generated by bacterial breakdown of urea or the ammonium isocyanate formed in aqueous solutions of urea. We found the course of the uremic syndrome could be accelerated in bilaterally nephrectomized dogs treated with peritoneal lavage solution containing either 1% urea or 0.015% potassium isocyanate. It is suggested that ammonium isocyanate formed from urea in aqueous solutions may contribute to the uremic syndrome via carbamylation reactions with free amino acids and proteins. Blood ammonia levels were not significantly elevated in any of the animals tested.

Grof J; Menyhart J; Somogyi J. (1972) **Effect of urea on the rate of urea cycle in-vitro**. Acta Physiol Acad Sci Hung 41: 3-4.

Hansen JA; Vilstrup H. (1985) **Kinetics of urea synthesis and alanine uptake by perfused rat livers**. Liver 5: 1-7. Eight livers of 200 g rats were isolated and perfused in a single pass system with a semi-synthetic medium to which alanine was added to concentrations from 0.5 to 15 mmol/l. In each liver, 4-5 sets of urea synthesis rate and alanine uptake rate at different alanine concentrations were measured. The urea synthesis rate in relation to the alanine concentration was compatible with substrate inhibition kinetics. The kinetic constants were (mean +/- SD): Vmax = 10.34 +/- 3.41 mumol urea-N/(min X 100 g b.w.), Km = 1.56 +/- 0.67 mmol/l, and Ki = 5.35 +/- 2.44 mmol/l. The alanine uptake rate followed Michaelis-Menten kinetics with the constants (mean +/- SD) Vmax = 7.51 +/- 1.68 mumol/(min X 100 g b.w.) and Km = 2.14 +/- 1.04 mmol/l. The constants were assessed by non-linear iterative regression analysis. Urea synthesis exceeded alanine uptake at alanine concentrations below 2 mmol/l, and was smaller at higher concentrations. In two experiments, alanine metabolites were measured. The glucose production rate in relation to the alanine concentration suggested substrate inhibition. At high alanine concentrations, ammonia, lactate and pyruvate were released by the livers. The results indicate that whole liver urea synthesis and gluconeogenesis is inhibited by high blood alanine concentrations, in contrast to alanine uptake.

Hewitt, S; Nicholas, TE. (1983) **Urea metabolism in isolated perfused lungs of the laboratory rat and of a desert rodent, Notomys alexis**. Comp Biochem Physiol A 74: 467-469. 1. Using the isolated perfused lung preparation we have demonstrated a low-activity ureolytic enzyme present in rodent lung tissue. The enzyme shares four characteristic features with jack bean urease (EC 3.5.1.5). 2. Ureolytic activity was inhibited by fluoride ions and methionine hydroxamic acid; using the latter inhibitor, the I50 value and maximum inhibition were similar to those reported for jack bean urease. The apparent Km for rat lung urease was similar to the plasma urea level. 3. The low level of urease activity in the rat lung and in that of Notomys alexis, a desert rodent, suggests that the enzyme is not involved in urea excretion, rather that pulmonary ammonia production may influence fluid balance at the alveolus.

Huang, T-C; Chen, D-H. (1991) **Kinetic study of urease-catalysed urea hydrolysis**. J Chem Technol Biotechnol 52: 433-444.

Husson A; Fairand A; Vaillant R. (1981) **Control of urea synthesis in fetal rat liver slices**. Biol Neonate 40: 5-6. Urea synthesis in fetal rats was studied using a liver slice system with ammonium chloride as nitrogen source. In term fetuses, the rate of urea formation increases with ammonium chloride concentration up to 100 microM and is enhanced by the addition of ornithine. The developmental pattern for urea synthesis is very similar to that found in the enzyme activity studies: urea production increases slightly during fetal life and then rises rapidly at birth. It is found that fetal liver has an absolute requirement for glucocorticosteroids to develop a normal urea synthesis. The accordance with argininosuccinate synthetase activity measured in liver homogenate is discussed.

Hutchinson DL.; Kelly WT.; Friedman EA.; et al. (1962) **The distribution and metabolism of carbon-labeled urea in pregnant primates**. J Clin Invest 41: 1745-1753. The synthesis of urea-C-13 from NaCN and its analysis in body fluids is described. Pregnant rhesus monkeys at term were subjected to routine operative procedures for the placement of catheters into maternal arteries, into placental vessels and amniotic fluid. A mixture of carbon-14 urea and tritium oxide was injected into one of these compartments and samples collected over a period of several hours. In two additional experiments urea-C-13 and C-14 were injected simultaneously into fetal and maternal bloodstreams. The specific activity curves for the first set of experiments indicated that radiourea is distributed in a general urea pool, that there is a rapid exchange of urea between maternal and fetal blood and an appreciably slower transfer to the amniotic fluid whose urea is derived from mother and fetus in an approximate ratio of 31. The exchange rate between maternal and fetal blood could be estimated as 1 to 2 millimoles of urea/kilogram fetus/hour. The maximal rate of endogenous urea production in the fetal organism amounts to less than 1/10 of the total placental exchange. ABSTRACT AUTHORS: Authors

Illnerova H. (1968) **Activity of urea cycle enzymes in the liver and urea excretion in the rat during development**. Physiol Bohemoslov 17: 70-76.

Ishida, H. (1963) **Studies on the Feto-Maternal Metabolism of Urea**. Tohoku J Exp Med 80: 205-217.

Jones ME. (1983) **Catalysts of the urea cycle**. Trans N Y Acad Sci 41: 77-82.

Juhr, NC; Franke, J. (1987) **Metabolism of Carbon-14-Labelled Urea in Conventional Germfree and Mono-Associated Rats**. Z Versuchstierkd 29: 3-4. This report deals with the utilization of C14-labeled urea in conventional, defined associated and germ-free rats. With conventional animals 71.44% of the administered C14-dose can be demonstrated in the exhaled air, 0.47% in organs and 27.35% in the urine. 1.04% were found in the intestinal and faecal contents. Animals mono-associated with Proteus mirabilis have about the same utilization rate (59.15, 0.34, 35.98, 2% resp.). In germfree animals 1.21% of the activity appeared in the exhaled air and shows a small part of non-enzymatic hydrolysis of urea. The excretion of 97.70% in the urine shows, that urea is absorbed from the intestine in germfree animals.

Juhr, NC; Franke, J. (1990) **[Metabolism of 14C-labeled urea in conventional and bacteria-free guinea pigs]. STOFFWECHSEL VON 14C-MARKIERTEM HARNSTOFF BEI KONVENTIONELLEN UND BAKTERIENFREIEN MEERSCHWEINCHEN**. Z Versuchstierkd 33: 123-127. This report deals with the utilization of 14C-labeled urea in antibiotically decontaminated, bacteria-free guinea pigs using intragastric resp. intraperitoneal administration route. With conventional animals 52% of the intragastrically administered 14C dose can be demonstrated in the exhaled air, 1.2% in organs and 41% in the urine. 1.8% were found in intestinal and faecal contents. In bacteria-free animals 29% of the activity appeared in the exhaled air, 52% in urine and 2% remain in the intestinal tract, feces and animal body. Intraperitoneal administration of 14C urea revealed 17% resp. 14% in the exhaled air, 72% resp. 66% in urine and 2% resp. 3% in intestinal tract, feces and animal body in conventional resp. bacteria-free guinea pigs.

Kashiwagura T; Deutsch CJ; Taylor J; et al. (1984) **Dependence of gluconeogenesis, urea synthesis, and energy metabolism of hepatocytes on intracellular pH**. J Biol Chem 259: 237-243. The relationship of intracellular pH to extracellular pH has been measured in suspensions of isolated hepatocytes at 25 degrees C. The internal pH was found to be a linear function of external pH and it changed by 0.45 pH unit per 1.0 unit change in external pH. The internal [H+] was equal to the external [H+] at approximately pH 7.1. Gluconeogenesis, urea synthesis, and oxidative phosphorylation showed different dependencies on the intracellular pH. Gluconeogenesis was the most sensitive to changes in

[H+] and it declined by 80% when the intracellular pH decreased from 7.1 to 6.9. Urea synthesis was less pH-dependent, decreasing by about 30% for the same change in the intracellular [H+] whereas respiratory rate showed very little dependence on pH at this temperature. Intracellular [ATP]/[ADP] decreased linearly from 8.5 to 1.5 as the intracellular pH increased from 6.8 to 7.6, while intracellular [Pi] was essentially constant at 3.2 nmol/mg of cells, wet weight. Cytochrome c became more reduced with increasing intracellular pH, from less than 10% at pH 6.8 to 35% at pH 7.7. The calculated free energy of hydrolysis of ATP was nearly independent of pH as was the free energy of electron transfer from the intramitochondrial NAD couple (calculated from the [acetoacetate]/[3-OH-butyrate] ratio) to cytochrome c.

Kekomaki M; Schwartz AL; Pentikainen P. (1970) **Rate of urea synthesis in normal and cirrhotic rat liver with reference to the arginine synthetase system**. Scand J Gastroenterol 5: 375-380.

Kleinman LI. (1966) **Excretion and synthesis of urea by the mammalian kidney**. Arq Port Bioquim 10: 73-83. A review of recent expts. concerning urea excretion with particular emphasis on 2 expts. in which it was found that renal urea synthesis occurred in 27% of the rats tested and that urea-to-inulin clearance ratios exceeded 1 in 21% of the expts. It is suggested that renal urea synthesis is an adaptive phenomenon, occurring in those animals which are limited in the ability to synthesize urea in the liver or to excrete urea by the kidney. After administration of a hepatotoxic agent, renal urea synthesis was observed in 4 of 5 animals who did not synthesize urea before the administration. 9 references.

Kramer JW; Freedland RA. (1972) **Possible rate-limiting factors in urea synthesis by the perfused rat liver**. Proc Soc Exp Biol Med 141: 833-835.

Krebs HA; Hems R; Lund P; et al. (1978) **Sources of ammonia for mammalian urea synthesis**. Biochem J 176: 733-737. The initial rate of incorporation of [15N]alanine into the 6-amino group of the adenine nucleotides in rat hepatocytes was about one-eighteenth of the rate of incorporation into urea. Thus the purine nucleotide cycle cannot provide most of the ammonia needed in urea synthesis for the carbamoyl phosphate synthase reaction (EC 2.7.2.5). On the other hand, contrary to the view expressed by McGivan & Chappell [(1975) FEBS Lett. 52, 1--7], the experiments support the view that hepatic glutamate dehydrogenase can supply the required ammonia.

Kretchmer, N; Hurwitz, R; Raiha, N. (1965) **Some aspects of urea and pyrimidine metabolism during development**. Biol Neonat 9: 187-196.

LeVeen, HH; LeVeen, EG; LeVeen, RF. (1994) **Awakenings to the pathogenicity of urease and the requirement for continuous long term therapy**. Biomed Pharmacother 48: 157-166. Urease is an enzyme found in plants and bacteria, but not mammals. It catalyzes the conversion of urea to carbon dioxide and ammonia. Ammonia shortens the life span of cells; and higher concentrations cause tissue necrosis and cytolysis. Twenty percent of total body urea is converted to ammonia by bacterial urease in the colon. Small injections of urease immunize animals by producing antiurease, a gamma globulin, which inactivates urease. Immunization eliminates the colonic conversion of urea to ammonia. Injection of urease produces ammonia intoxication making immunization hazardous. Although previously impossible, a non enzymatic urease antigen was synthesized by covalently bonding jack bean urease with glutaraldehyde. This antigen stimulated the production of antiurease that inactivates native urease. Helicobacter pylori, a potent urease producer, has been implicated in peptic ulcer, gastritis and other inflammatory bowel lesions. The pathogenicity of H pylori is dependent on its urease production. Immunization to urease can render H pylori non pathogenic. Cirrhotics develop encephalopathy and hyperammonemia because their livers fail to convert all the ammonia in portal venous blood to urea and

collaterals develop by passing the liver. Colonic ammonia increases the turnover rate of colonic mucosa. Ammonia absorbed into the portal venous system is transported to the liver where it is reconverted to urea. Absorbed ammonia adversely influences liver function. Infections with urease producing organisms destroy the renal parenchyma and produce struvite stones. Urease immunization aids colonic healing and prevents uremic colitis. Absorbed ammonia is a noxious influence on the liver. Animals immunized to urease regenerate the liver faster and are less susceptible to hepatotoxins. Immunization to urease ameliorates cirrhosis. Proteus and other urease producers become non toxic and do not damage the renal parenchyma. Urease is responsible for the pathogenicity of infections with urease producing organisms. Immunization to urease renders urease producing organisms non pathogenic.

Long, CL; Jeevanandam, M; Kinney, JM. (1978) **Metabolism and recycling of urea in man**. American J Clin Nutr 31: 1367-1382. The rate of breakdown and reutilization of urea was estimated in 5 normal and 2 septic patients using 15N- and 13C-labelled ureas. The labelled molecules of the [15N] urea dose were distinguished from the labelled molecules of the recycled urea by analysing in a mass spectrometer the isotopic nitrogens produced when the recrystallized urine urea was treated with hypobromite solution. In a normal subject with normal intake of N only 80% of the produced urea was excreted in urine and the rest was endogenously degraded; 70% of the N and 63% of the carbon of the degraded urea were returned to the urea pool. On a nitrogen-free diet or after neomycin treatment with normal diet in the normal subject, the extent of urea splitting was reduced. In the septic patients, breakdown and recycling of urea were almost eliminated. It seems that the rate of endogenous catabolism of urea depends mainly on the activity of the gut flora which may be affected by dietary intake and clinical status of the subject. The method could be applied to the study of urea dynamics in different physiological and pathological conditions.

Martinson, E; Tyakhepil'D, L; Lind, A; et al. (1961) **Transformations of urea in gastric mucosa**. Biokhimiia [Trans] 26: 1-6. The ammonia content of gastric venous blood rises after introduction of urea solution into the clamped stomach of a dog than when ammonium salts are introduced, under comparable experimental conditions. It is hence concluded that hydrolysis of urea, catalyzed by intracellular urease, occurs in the gastric mucosal cells, with formation of urea. Increase in the glutamine content of gastric venous blood indicates that the ammonia formed from urea reenters the cycle of biochemical processes in the secretory cells of the stomach. A comparison of the rise in the ammonia content of distilled water and of neutral or strongly acid urea solutions introduced into the stomach, following intravenous injection of ammonium salts, leads to the conclusion that the ammonia is released from the gastric mucosa, but does not originate from the action of bacterial urease in the lumen of the stomach. Hydrolysis of urea provides a source of ammonia in the gastric mucosa; the reaction is of local significance only, and it enters into the biochemistry of gastric secretion, as is indicated by its intensification following histamine administration. ABSTRACT AUTHORS: Authors

McLean, RJ; Nickel, JC; Cheng, KJ; et al. (1988) **The ecology and pathogenicity of urease-producing bacteria in the urinary tract**. Crit Rev Microbiol 16: 37-79. Urease activity is a physiological function of many bacteria that enables these organisms to utilize urea as a source of nitrogen. The association of ureolytic bacteria with human or animal hosts varies widely from a commensal relationship as demonstrated with skin microflora, a symbiotic relationship in the gastrointestinal tract, to a pathogenic relationship in the urinary tract. Since similar or identical species of bacteria such as Staphylococcus aureus are found in all three environments, the effect of urease activity on the host must be solely a function of the environment of these organisms. In this review, the importance of urease to bacteria is discussed, identifying the gastrointestinal tract as a major reservoir of ureolytic bacteria and investigating the urinary tract environment and the infectious struvite stone production that often accompanies urease-

producing bacteria there. Finally, an infection model is presented which explains the development and growth of these urinary calculi and their remarkable persistence in spite of modern urological treatments.

Meakins, TS; Jackson, AA. (1996) **Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets**. Clin Sci 90: 215-225. 1. Urea kinetics were measured in six healthy men using prime/intermittent oral doses of [15N15N] urea, after five days consuming one of four diets which varied in their nitrogen content: a reference diet (REF, 70 g of protein and 11.2 g of N); a low-protein diet (LP, 30 g of protein and 4.8 g of N); a low-protein diet with 6.9 g of urea added (LP-U1, 30 g of protein and 8 g of N); a low-protein diet with 13.7 g of urea added (LP-U2, 30 g of protein and 11.2 g of N). 2. Apparent nitrogen balance on the REF diet was significantly better than on the LP or the LP-U1 diets. The addition of the higher level of urea in the LP-U2 diet enhanced apparent nitrogen balance compared with the LP or LP-U1 diets, and was not different to apparent nitrogen balance on the REF diet. 3. On the LP, LP-U1 and LP-U2 diets, the rate of endogenous urea production was not different, and was about 60% of that on the REF diet, a statistically significant difference. The addition of a dietary supplement of urea increased the rate of urea appearance in the urea pool in direct relation to the dose of urea taken. There was no difference in the rate of appearance between the REF and LP-U2 diets, for both of which the rate of appearance was significantly greater than on the LP diet. 4. The excretion of urea in urine on the LP diet was 62% of that on the REF diet, a significant difference. There was no significant difference in the rate of urea excretion between the REF, LP-U1 and LP-U2 diets. 5. The rate of urea hydrolysis by the colonic microflora on the REF diet was more than twice that on the LP or LP-U1 diets. Supplementation with urea at the higher level, LP-U2, significantly increased hydrolysis to the same level as on the REF diet. Most of the nitrogen derived from urea hydrolysis was retained in the metabolic pool (> 80%), with no difference in the rate of retention between the REF and LP-U2 diets, both greater than the LP or LP-U1 diets. 6. The dietary supplements of urea increased the size of the body urea pool significantly. Renal clearance of urea was highest on the REF diet and decreased 13-29% on the low-protein diets. Bowel clearance was highest on the REF diet and decreased 46-55% on the low-protein diets. Neither urinary excretion of urea nor urea hydrolysis in the bowel were related simply to the concentration of urea in blood. Urea hydrolysis related most closely to the rate of appearance of urea in the urea pool. 7. The salvage of urea nitrogen was increased on the highest level of supplementation, but the overall sensitivity of the system was low, suggesting that other factors might be limiting for effective urea hydrolysis and the salvage of urea nitrogen.

Menyhart J; Grof J. (1977) **Urea as a selective inhibitor of argininosuccinate lyase**. Eur J Biochem 75: 405-409. The effect of urea on various ornithine cycle enzymes has been investigated. It was demonstrated that argininosuccinate lyase was the only ornithine cycle enzyme inhibited by urea in a competitive manner. Based on the data presented the possible role of urea in maintaining a physiological range of intracellular and extracellular urea concentration by controlling hepatic ureogenesis was discussed.

Moran, BJ; Jackson, AA. (1990a) **15N-urea metabolism in the functioning human colon: luminal hydrolysis and mucosal permeability**. Gut 31: 454-457. The biopsy channel of the colonoscope was used in a novel approach to the study of in vivo colonic nitrogen metabolism in 12 subjects. A tracer dose of 15N15N-urea was placed in the caecum in six and distal to the splenic flexure in six. The urine and stool were collected for 72 hours and isotopic enrichment was measured in a mass spectrometer. A similar proportion of the dose was recovered in the urine as 15N15N-urea from the right colon, 6%, as was recovered from the left, 4%, showing that the urea was absorbed intact. Urinary 15N14N-urea from the right colon was 18% of the dose compared to 13% from the left colon. This represents urea that has been hydrolysed and absorbed as ammonia. Less than 4% of the dose was recovered in the stool. The greatest proportion of the label, 74% from the right and 82% from the left, could not be accounted for in

the urine or the stool and is presumed to have entered the metabolic pool of nitrogen. We conclude that; the colon is permeable to urea, intraluminal hydrolysis occurs and that urea nitrogen enters the metabolic pool of nitrogen in functionally significant quantities.

Moran, BJ; Jackson, AA. (1990b) **Metabolism of 15N-labelled urea in the functioning and defunctioned human colon**. Clin Sci 79: 253-258. 1. The luminal metabolism of urea was studied using double-labelled urea ([15N2]urea) which was placed in the lumen of the colon through a colostomy. The recovery of label was measured as [15N2]urea or [14N, 15N]urea in urine and as 15N in stool. 2. Five patients with a loop colostomy allowed a comparison of the right functioning colon with the left defunctioned colon in the same individual. Five subjects with a left end-colostomy enabled a comparison of the right with the left functioning colon. 3. A significantly greater proportion of labelled urea was recovered as [15N2]urea in the urine when the dose was placed in the left defunctioned colon (29%) compared with either the left or the right functioning colon (9 and 4%, respectively). This is interpreted as being a result of a decrease in the bacterial activity and concomitant urea hydrolysis in the defunctioned colon. 4. On average more than half of the label was retained in the body, regardless of whether the urea was placed in the functioning or the defunctioned colon, on the left or on the right. 5. The data confirm that the colon is permeable to the intact urea molecule. Intraluminal urea is readily hydrolysed in the functioning colon. A large proportion of the nitrogen released by urea hydrolysis may be retained within the metabolic nitrogen pool of the host. There are significant differences in the handling of urea nitrogen in the defunctioned colon relative to the functioning colon.

Mullins Von Dreele M; Banks RO. (1985) **Urea synthesis in the canine kidney**. Ren Physiol 8: 73-79. The ability of the kidney to synthesize urea is of interest and some controversy. We tested the hypothesis that low plasma urea concentrations would stimulate renal urea synthesis, thereby enhancing urinary concentrating ability. We defined a ratio of specific activity of plasma/specific activity of urine (SA ratio) greater than 1.0 as indicative of de novo intrarenal urea synthesis. We studied dogs on 3 dietary regimens: (1) commercial chow ad libitum; (2) restricted rations of the same chow; (3) protein-free diet ad libitum with vitamin/mineral supplement. A significant inverse relationship between plasma urea concentration and SA ratio was seen only in the ration-restricted group, despite the fact that plasma urea concentrations in this group were highest of the three groups. In addition, dogs in this group were more likely to demonstrate renal urea synthesis than dogs in the other groups. Thus, the dog kidney is capable of de novo urea synthesis, but a reduced plasma urea concentration does not necessarily stimulate this metabolic activity. Other factors in addition to the plasma urea concentration appear to be involved in the regulation of renal urea synthesis.

Neuhofer W; Muller E; Burger-Kentischer A; et al. (1998) **Pretreatment with hypertonic NaCl protects MDCK cells against high urea concentrations**. Pflugers Arch 435: 407-414. In antidiuresis, the cells of the renal medulla are exposed to high extracellular concentrations of NaCl and urea. Since urea equilibrates with the intracellular compartment and is known to perturb intracellular macromolecules, high urea concentrations may well disturb the structure and function of cell proteins. Two types of organic substances are believed to counteract the adverse effects of high intracellular urea concentrations: specific organic osmolytes of the trimethylamine family [betaine and glycerophosphorylcholine (GPC)], which accumulate in renal medullary cells during prolonged periods of antidiuresis and cytoprotective heat shock proteins (HSPs), the tissue content of two of which (HSPs 27 and 72) is much higher in the inner medulla than in the iso-osmotic renal cortex. To evaluate the contribution of trimethylamines and HSPs to cytoprotection in the presence of high urea concentrations, the effect of HSP induction and osmolyte accumulation prior to exposure to high urea concentrations was examined in Madin-Darby canine kidney (MDCK) cells. Accumulation of organic osmolytes and synthesis of HSP27 and HSP72 was initiated by hypertonic stress (increasing the osmolality of the

medium from 290 to 600 mosmol/kg H2O by NaCl addition). Control, non-conditioned cells remained in the isotonic medium for the same period. Upon subsequent exposure to an additional 600 mM urea in the medium for 24 h, 90% of the osmotically conditioned cells but only 15% of non-conditioned cells survived. The HSP72 and trimethylamine contents of the NaCl-conditioned MDCK cells, but not HSP27 content, correlated positively with cell survival. To separate the effects of organic osmolytes and HSP72, chronically NaCl-adapted MDCK cells were returned to isotonic medium for 1 or 2 days, so depleting them of trimethylamine osmolytes. HSP72, with its longer half life, remained elevated. Subsequent exposure of these cells to 600 mM urea in the medium resulted in about 80% survival. These results suggest that in MDCK cells and probably in the renal medulla, HSP72 and perhaps additional protective factors contribute substantially to the resistance against high urea concentrations.

O'Connor JE; Jorda A; Grisolia S. (1985) **Acute and chronic effects of carbamyl glutamate on blood urea and ammonia**. Eur J Pediatr 143: 196-197. Carbamyl glutamate injected into normal rats produced no change in blood urea levels. Rats fed a high-protein diet or starved for 48 h had increased blood urea. Carbamyl glutamate injection induced a further increase in the levels of urea in their blood. Also, carbamyl glutamate administered in the drinking water of normal mice produced an increase in blood urea, which was accompanied by a decrease in blood ammonia. The application of these findings to the treatment of urea cycle enzymopathies is discussed.

Phromphetcharat V; Jackson A; Dass PD; et al. (1981) **Ammonia partitioning between glutamine and urea: interorgan participation in metabolic acidosis**. Kidney Int 20: 598-605. The distribution of precursor nitrogen between urea and glutamine was studied in control and acidotic rats. Acidosis, either acutely induced with hydrochloric acid or chronically induced with ammonium chloride, resulted in a rise in ammonia and a fall in urinary urea excretion; the percent of urinary nitrogen excreted as ammonia rose from 3.5 +/- 0.4 and 4.9 +/- 0.5 in fed and pair-fed controls to 25.9 +/- 3.9 and 37 +/- 5 in acidosis induced by hydrochloric acid and ammonium chloride. Hepatoportal vein urea concentration differences were significantly reduced, whereas glutamine concentration differences were significantly elevated, consistent with a shift of nitrogen from ureagenesis to glutamine; alanine and ammonia concentration differences were significantly decreased and increased respectively in the acidotic animals, suggesting that former supported urea synthesis whereas ammonia may preferentially support glutamine synthesis. Evidence of a feed-forward involvement of the gut in influencing hepatic nitrogen distribution was suggested by an increased ammonia and decreased alanine release in acidotic rats. Bilateral ureteral ligation was performed on control and acidotic rats to determine the fate of the redirected urinary ammonia. Ammonia did not accumulate in the blood, rather it was initially incorporated into glutamine, elevating the plasma level, and then it was subsequently deposited into urea . The shift of nitrogen back into urea in acidotic animals was confirmed by the greater postligation urea production rates supported by hepatic uptake of both alanine and glutamine. These results are discussed in terms of interorgan participation involving the liver, gut, and muscle in the partitioning of nitrogen between glutamine and urea.

Prabhakar SS; Zeballos GA; Montoya-Zavala M; et al. (1997) **Urea inhibits inducible nitric oxide synthase in macrophage cell line**. Am J Physiol 273: C1882-8. Macrophage dysfunction is considered an important contributory factor for increased propensity of infections in uremia. Because nitric oxide (NO) is believed to be an effector molecule of macrophage cytotoxicity, we propose that the dysfunction may be related to impaired NO synthesis. To verify this hypothesis, we evaluated macrophage NO synthesis in the presence of urea, a compound that accumulates in renal failure and is believed by some to be a uremic toxin. Macrophages (RAW 264.7 cells) were incubated with bacterial lipopolysaccharide to induce NO synthesis, whereas the test groups had various concentrations of urea in addition. NO synthesis was measured by assaying the supernatant for nitrites and nitrates by chemiluminescence. We

observed that urea consistently produced a dose-dependent reversible inhibition of inducible NO production in macrophages, whereas parathormone, another toxin retained in uremia, had no such inhibitory effects. Further studies revealed that mRNA for inducible NO synthase was not inhibited by urea. We thus conclude that urea inhibits inducible NO synthesis in macrophages by a posttranscriptional mechanism and that this may be important in macrophage dysfunction of uremia.

Prior, RL; Visek, WJ. (1972) **Effects of urea hydrolysis on tissue metabolite concentrations in rats**. Am J Physiol 223: 1143-1149.

Prior, RL; Visek, WJ. (1973) **Effects of urea in rats deprived of arginine**. J Nutr 103: 1107-1111.

Sadasivudu B; Rao TI. (1976) **Studies on functional and metabolic role of urea cycle intermediates in brain**. J Neurochem 27: 785-794. The distribution of argininosuccinate synthetase, argininosuccinase and arginase, and the synthesis of urea in cerebullum, cerebral cortex and brain stem were studied. Cerebral cortex had high levels of argininosuccinate synthetase and argininosuccinase, and a high ability to synthesize urea from aspartic acid and citrulline. Of the 3 regions, cerebullum had the highest arginase activity. The activities of the enzymes transamidinase and ornithine aminotransferase in the metabolism of arginine and ornithine in pathways other than urea formation were studied in 3 regions of the rat brain. The activity of creatine phosphokinase in all regions was the same: carbamylphosphatase activity was highest in cerebullum. Cerebral cortex had a high activity of aspartic acid transcarbamylase. The brain stem, among the 3 regions, had the lowest activities of glutamine synthetase and glutaminase. The activities of these enzymes in the different regions were discussed in relation to urea production and the utilization of the urea cycle intermediates. I.p. injection of high amounts of citrulline brought about a rise in the glutamine synthetase activity of cerebellum and brain stem and a rise in ornithine aminotransferase in cerebral cortex and liver. These results were discussed in relation to the mechanism of action of citrulline in alleviating the toxicity in hyperammonemic states.

Saheki T. (1972) **[The studies on regulatory conditions of urea synthesis using isolated perfused rat liver. 2. Urea synthesis in the rat liver subjected to different dietary conditions.]**. Shikoku Acta Med 28: 292-298. 2. Urea synthesis was studied in perfused livers isolated from rats weighing 150 to 220 g which had been given diets containing different amounts of casein. Urea formed after the addition of 600 mu moles ammonium chloride to the perfusion fluid in preparations from rats given 5, 10, 40 or 70% casein or deprived of food from 20 to 24 h was 108.4, 198.3, 276.7, 293.7 and 281.3 mu moles, respectively. When 150 mu moles ornithine and 1200 mu moles acetylglutamate were added with 600 mu moles ammonium chloride to the perfusion fluid in the preparation from rats given 5% casein, urea synthesis increased to 189.5 mu moles. When 12 mmoles ammonium chloride was added with 150 mu moles ornithine and 1200 mu moles acetylglutamate to the perfusion fluid in the preparation from rats given 70% casein complete conversion of the ammonia or urea occurred. The ratio of rate of urea formation to liver ornithine transcarbamylase activity was lower in preparations from rats given 5% casein than those from rats given 70% casein. The ratio increased to a similar value in preparations from both groups when 2.5 mM ornithine or 20 mM acetylglutamate was added to the perfusion fluid. In perfusate of preparation from rats given 5% casein, alanine, aspartate and glutamine increased when 600 mu moles ammonium chloride were added to the perfusion fluid. Those amino acids also rose in the liver of rats given 5% casein. Similar but less marked changes occurred in the perfusates of preparations from rats given 70% casein. (From summary.).

Saheki T. (1972) **[The studies on regulatory conditions of urea synthesis using isolated perfused rat liver. 1. Urea synthesis from ammonia and glutamine, and its regulatory factors.]**. Shikoku Acta Med 28: 284-291. 1. When livers of rats weighing from 130 to 180 g were isolated and perfused at 20

ml/min for 30 min in a recirculating system with solutions containing different amounts of ammonium chloride, half maximum rate of urea synthesis occurred when ammonium concentration was 0.7 mM and rate was independent of ammonia concentration at concentrations between 5 and 20 mM. When 10 mmoles ammonium chloride/litre perfusion fluid was added, subsequent addition of aspartate, fumarate, malate or pyruvate 10 mmoles/litre increased the rate of urea formation/g liver by 56, 51, 90 and 30%, respectively. When 2.5 mmoles ornithine/litre was added to perfusion fluid containing 10 mM ammonium chloride, rate of urea formation was increased by 127%. Addition of 10 or 20 mmoles acetylglutamate/litre increased rate of urea formation from 10 mM ammonium chloride by 44 and 147%. The effects of ornithine and aspartate and of ornithine and acetylglutamate but not that of aspartate and acetylglutamate were additive. Acetylglutamate had a greater effect when it was added before ammonium chloride. Urea was also formed when liver was perfused with glutamine but the rate was not significantly affected when ornithine, malate or acetylglutamate was added to the perfusion fluid. Half maximum rate of disappearance of glutamine occurred at a concentration of 5 mM and the optimum pH for glutamine removal was 7.7. (From summary.).

Sanzey B; Ullmann A. (1976) **Urea, a specific inhibitor of catabolite sensitive operons**. Biochem Biophys Res Commun 71: 1062-1068.

Schimke, RT. (1962) **Adaptive characteristics of urea cycle enzymes in the rat**. J Biol Chem 237: 459-468. The effects of wide variation of dietary protein consumption on enzyme activities of the pathway directly associated with urea synthesis, i.e. carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, arginino-succinate cleavage enzyme, and arginase, have been determined. The total liver content of each of these enzymes was found to be proportional to the daily consumption of protein. Characterization of purified arginase and ornithine transcarbamylase from rat livers with widely differing activities indicated that observed differences were due to dif-ferences in actual content of specific enzyme protein rather than due to effects of activators, inhibitors, or alterations in the kinetic properties of enzyme molecules. It is proposed that the changes in levels of urea cycle enzymes associated with alterations in protein intake represent an example of adaptation in a mammalian system which involves synthesis and degradation of specific enzyme protein. ABSTRACT AUTHORS: Author

Schunemann, C; Klocke, B; Arndt, J; et al. (1987) [**Studies of intestinal urea metabolism in the dog] UNTERSUCHUNGEN ZUM INTESTINALEN HARNSTOFFUMSATZ BEIM HUND**. Bericht des 17. Kongresses der Deutschen Veterinarmedizinischen Gesellschaft, Bad Nauheim, 1.-4. April 1-4, 1987, No. 17, pp. 424-429. Relations between postprandial plasma urea, urea and ammonia concentrations in ileum, colon and faeces were studied in 3 colon fistulated and 2 ileum fistulated dogs and 5 intact dogs given crude protein 2.6 to 14.3 g/kg0.75 daily. In a further experiment urea 50 mg kg-0.75 min-1 was infused with saline with or without previous antibiotics. Postprandial plasma urea increased linearly with increasing crude protein intake and postprandial urea in ileal digesta was positively correlated with plasma urea. With increasing plasma urea concentrations, urea and ammonia content of stomach and colon increased.

Singer, MA. (2003) **Do mammals, birds, reptiles and fish have similar nitrogen conserving systems?** Comp Biochem Physiol B Biochem Mol Biol 134: 543-558. Comparative physiological studies are a powerful tool for revealing common animal adaptations. Amino acid catabolism produces ammonia which is detoxified through the synthesis of urea (mammals, some fish), uric acid (birds), or urea and uric acid (reptiles). In mammalian herbivores and omnivores, urea nitrogen is salvaged by a series of steps involving urea transfer into the intestine, microbial mediated urea hydrolysis with synthesis of amino acids utilizing the liberated ammonia and transfer of the amino acids back to the host. A similar series of steps occur in omnivorous/granivorous and herbivorous birds, although in this case urine, containing uric

acid, is refluxed directly into the intestine where microbes degrade the uric acid and utilize the liberated ammonia for amino acid synthesis. These amino acids are transferred back to the host. In reptiles and ureotelic fish not all of these steps have been experimentally confirmed. Reptiles like birds, reflux urine into the intestine where it is exposed to the microflora. However, the capacity of these microbes to breakdown the uric acid and urea and utilize ammonia for amino acid synthesis has not been documented. Ureotelic fish transfer urea into the intestine where urease (presumably of bacterial origin) hydrolyzes the urea. However, the amino acid synthesizing capacity of the intestinal microflora has not been studied. The series of steps, as outlined, would define the prevailing nitrogen conservation system for herbivores and omnivores at least. However, it would appear that some animals, in particular the fruit-eating bat and perhaps the fruit-eating bird, may have evolved alternative, as yet uncharacterized, adaptations to a very limited nitrogen intake.

Snodgrass PJ; Lin RC; Muller WA; et al. (1978) **Induction of urea cycle enzymes of rat liver by glucagon**. J Biol Chem 253: 2748-2753. All five urea cycle enzymes of rat liver increased in activity 48 h after subcutaneous administration of crystalline zinc glucagon to male rats and remained elevated after 7 days of continuous glucagon infusion. The maximum ratios of enzyme activities over those of controls were 2.0 for carbamyl phosphate synthetase, 1.3 for ornithine transcarbamylase, 2.7 for argininosuccinate synthetase, 3.2 for argininosuccinase, and 2.2 for arginase. Actinomycin D or puromycin prevented these responses to glucagon. The increase in arginase activity after zinc glucagon treatment was matched by an increase in immunoprecipitable enzyme. All five enzymes were induced by physiological plasma levels of glucagon. Tube feeding of casein hydrolysate for 2 days increased all five enzyme activities 1.5- to 2.2-fold and resulted in plasma glucagon levels similar to those required for induction by exogenous glucagon. Thus, glucagon is an inducer of the entire urea cycle in rat liver and plays a role in the induction of the cycle by protein feeding.

Spanel, P; Turner, C; Wang, T; et al. (2006) **Generation of volatile compounds on mouth exposure to urea and sucrose: implications for exhaled breath analysis**. Physiol Meas 27: N7-17. The increase in ammonia and ethanol in the exhaled breath stream following mouthwashes by aqueous solutions of urea and sugar (sucrose), respectively, has been investigated by analysing exhaled breath in real time using selected ion flow tube mass spectrometry, SIFT-MS. It is shown that the measured levels of these compounds in the stream of exhaled breath can be much greater than the endogenous levels originating at the alveolar boundary. Thus, it is concluded that without careful preparation, mouth production of these compounds, and other compounds as yet unidentified, can seriously compromise the quantification of truly endogenous trace compounds present in blood and in the alveolar breath, as required for clinical diagnosis, and can probably introduce additional compounds into the breath stream that could seriously mislead breath analysis. The concentrations of both the urea and sucrose solutions used to enhance the ammonia and ethanol levels were larger than normally present in food and drinks and so in most situations such severe enhancements will not occur.

Tanaka A; Chance B; Quistorff B. (1989) **A possible role of inorganic phosphate as a regulator of oxidative phosphorylation in combined urea synthesis and gluconeogenesis in perfused rat liver. A phosphorus magnetic resonance spectroscopy study**. J Biol Chem 264: 10034-10040. Metabolic control of oxidative metabolism was studied in perfused rat liver by means of phosphorus magnetic resonance spectroscopy. Oxygen consumption, ATP, and Pi were measured with different rates of gluconeogenesis and urea synthesis by varying concentrations of the substrates in the perfusate. Five levels of oxygen consumption (VO2) were obtained: an average control value of 1.94 +/- 0.14 and 2.93 +/- 0.25, 3.29 +/- 0.46, 3.85 +/- 0.26, and 4.18 +/- 0.56 mumol/min/g liver (mean +/- S.D., n = 6). The corresponding ATP concentrations were 2.51 +/- 0.20, 2.39 +/- 0.08, 2.24 +/- 0.09, 2.13 +/- 0.12, and 1.91 +/- 0.13 mM. Pi increased stoichiometrically with the decrease in ATP. Free Pi (Pif) was calculated

as NMR-visible Pi in control plus -delta ATP (1.94 mM + (-delta ATP]. The kinetic relationship of oxidative phosphorylation as a function of Pif followed a Michaelis-Menten type of equation: VO2 = 5.55/(1 + 0.24/[(Pif] - 1.81]. The observed Km value for Pi of 0.24 mM approximates the reported Km value in isolated mitochondria of 1 mM. The free Pi concentration of 1.94 mM is in the range of the Km value, while the free ADP concentration of 200 microM exceeds the Km value of 20 microM. Therefore, it is suggested that Pi play a major role in the regulation of mitochondrial oxidative phosphorylation in combined urea synthesis and gluconeogenesis.

Tserng, KY; Kalhan, SC. (1982) **Gas chromatography/mass spectrometric determination of [15N]urea in plasma and application to urea metabolism study**. Anal Chem 54: 489-491.

Tujioka K; Lyou S; Sano A; et al. (2004) **Changes in tissue protein synthesis are involved in regulating urea synthesis in rats given proteins of different quality**. J Nutr Sci Vitaminol 50: 356-361. The purpose of present study was to determine whether the regulation of urea synthesis is mediated through changes in supply of amino acids by protein synthesis and whether the concentration of ammonia, or activities of amino acid catabolizing enzymes, regulate urea synthesis when the dietary protein quality is manipulated. Experiments were done on three groups of rats given diets containing 10 g gluten, 10 g casein or 10 g whole egg protein/100 g for 10 d. The urinary excretion of urea, and the liver concentrations of glutamate, serine and alanine increased with a decrease in quality of dietary protein. The fractional and absolute rates of protein synthesis in tissues declined with the decrease in quality of dietary protein quality. The ammonia concentration in plasma and liver, and activities of hepatic amino acid catabolizing enzymes was not related to urea excretion under these conditions. These results suggest that the lower protein synthesis seen in tissues of rats given the lower quality of protein is likely to be one of the factors to increasing the supply of amino acids and stimulating urea synthesis.

Visek, WJ. (1962) **Studies on urea hydrolysis in birds and mammals**. Am J Vet Res 23: 569-574.

Visek, WJ. (1972) **Effects of urea hydrolysis on cell life-span and metabolism**. Fed Proc 31: 1178-1193.

Wohlrab W. (1976) **[Normalization process in the epidermis following its previous exposure to urea]**. Dermatol Monatsschr 162: 585-589.

Wrong, OM; Vince, A. (1984) **Urea and ammonia metabolism in the human large intestine**. Proc Nutr Soc 43: 77-86.

Zimber, A. (1979) **Ammonia toxicity, urea hydrolysis and cellular turnover in animals**. Refu Vet 35: 181-193.

Zhou, WR; Gao, JH; Zhang, Y; et al. (1999) **Studies on the mechanism of absorption, digestion and utilization of 15N- urea in rabbits: III. Utilization of urea in diet for coprophagy-prevented rabbit**. Jiangsu Journal of Agricultural Sciences 15: 177-181. Six 2.5-kg New Zealand male rabbits were selected to study the metabolism of urea-N being utilized in rabbits using the 15N-urea tracing technique. In order to prevent coprophagy, each rabbit had a collar round its neck. The basal diet which contained 16.01% CP was fed at 120 g/day with additive 1% 15N-urea (1.2 g). 15N balance in coprophagy-prevented rabbits was 38.1 mg/day, 7.0% of the 15N-urea uptake, and the utilization coefficient of 15N was 8.0%, about 39% of that of control rabbits. Preventing coprophagy inhibited utilization of 15N in the diet. Protein-N in the stomach and intestine of coprophagy-prevented rabbits was 9.8 and 14.7%, 14 and 23% of control rabbits, respectively. It could be inferred that

microorganisms in control rabbits played an important role in transformation of 15N-urea to protein-N. From 3 studies it is concluded that: rabbits could utilize urea in their diets; activity in the caecum and coprophagy were the physiological base for utilization of urea; microorganisms were the key in utilization of non-protein N.

3.3.1 Effect of external factors (diet, exercise, health, etc.)

Bazhenov, SV; Stepanyuk, VD. (1970) **Metabolism of ammonia and glutamine in brain tissue during urea toxicosis**. Visn Silskogospod Nauki 13: 96-100. Toxicosis caused by feeding fodder containing synthetic urea supplements was due to the upset of the ammonia-glutamine equilibrium in cerebral tissue.

El-Khoury, AE; Borgonha, S; Pereira, PCM; et al. (1999) **24-Hour leucine and urea kinetics at a low protein intake in healthy young men**. FASEB J 13: 17-21.

Forslund, A; El-Khoury, AE; Olsson, R; et al. (1995) **Effect of moderate physical activity upon protein and energy homeostasis in healthy adult humans: 24H studies with leucine and urea stable isotope tracers**. FASEB J 9: 9-13.

Forslund, AH; Hambraeus, L; El-Khoury, AE; et al. (1998) **24h Studies on whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults**. FASEB J 12: 18-22.

Forslund, AH; Hambraeus, L; Olsson, RM; et al. (1998) **The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults**. Am J Physiol 275: E310-320. In healthy adult men adapted to a diet/exercise regimen for 6 days, the effects of small, frequent meals supplying daily protein intakes of 1 (n = 8) or 2.5 g . kg-1 . day-1 (n = 6) on leucine oxidation, urea production, and whole body protein synthesis (PS) and degradation (PD) have been compared with the use of a 24-h continuous intravenous [13C]leucine and [15N,15N]urea infusion protocol. Two 90-min periods of exercise (approximately 50% maximal O2 consumption) were included during the fasting and the fed periods of the 24-h day. Subjects were determined to be at approximate energy, nitrogen, and leucine balances on both diets. Increased protein intake raised the urea production rate; the absolute rate of urea hydrolysis was the same on both diets. When the first-pass splanchnic uptake of leucine was taken to be 25% of intake, PS was stimulated by feeding (after an overnight fast) at both protein intake levels ($P < 0.05$ and $P < 0.01$), whereas PD declined significantly ($P < 0.01$) at both protein levels. Protein gain at a high protein intake appears to be the result of both a stimulation of PS and a marked decline in PD, whereas at a less generous intake, the gain appears to be a result of a fall in PD with a less evident change in PS. Exercise moderately decreased PS during and/or immediately after exercise at each protein level, and there was a postexercise-induced increase ($P < 0.01$) in PD, which was more dramatic when feeding was at the higher protein intake level.

Hansen, BA; Vilstrup, H. (1985) **Increased intestinal hydrolysis of urea in patients with alcoholic cirrhosis**. Scand J Gastroenterol 20: 346-350. Fourteen patients with biopsy-proven alcoholic liver cirrhosis in a clinically stable phase but with compromised liver function entered the study, together with 10 control persons. All had normal creatinine clearance, and none received antibiotics or hormones. They ingested a diet containing 1 g of protein/kg body weight daily during the study. The fractional intestinal loss of newly synthesized urea , determined by a 14C-urea tracer method, was increased from 0.17 +/- 0.08 in controls to 0.26 +/- 0.08 in cirrhotics (mean +/- SD, P less than 0.02). Urea nitrogen synthesis rate, determined as urinary excretion rate, corrected for accumulation in the total body water and for

fractional intestinal loss, was the same in controls and cirrhotics (26.1 +/- 3.8 and 22.1 +/- 6.8 mmol/h, respectively). The patients with cirrhosis had a significantly greater nitrogen balance than the control group (12.5 +/- 7.0 mmol/h versus 7.0 +/- 5.9 mmol/h; P less than 0.05). Furthermore, there was a positive correlation between intestinal loss and blood urea nitrogen concentration (r = 0.68, P less than 0.01) in patients with cirrhosis but not in controls. The increased endogenous ammonia load of cirrhotics corresponds to an extra protein intake of 30-35 g/day. In patients with cirrhosis prophylactic treatment with, for example, lactulose is rational before reduction in dietary protein.

Harding, JJ. (1992) **Cyanate derived from urea in uraemia**. Lancet 339: 492.

Kalhan, SC; Tserng, KY; Gilfillan, C; et al. (1982) **Metabolism of urea and glucose in normal and diabetic pregnancy**. Metabolism 31: 824-833.

Kesteloot, HE; Joossens, JV. (1993) **Relationship between dietary protein intake and serum urea, uric acid and creatinine, and 24-hour urinary creatinine excretion: the BIRNH Study**. J Am Coll Nutr 12: 42-46. Relationships between dietary protein intake of a large free-living group and serum creatinine (Cr), urea and uric acid levels have been studied, based on data obtained from the BIRNH (Belgian Interuniversity Research on Nutrition and Health) study. Highly significant correlations were found in both sexes for total protein, as well as for animal and vegetable protein intake. In a subgroup, the relationship between dietary protein intake and 24-hour urinary Cr excretion has also been studied. The range of +/- 2 SD of the mean for total protein and animal and vegetable protein daily intakes resulted in a calculated range of 24-hour Cr excretion of 430, 317 and 209 mg/24 hours in men and of 192, 169 and 125 mg/24 hours in women, respectively. The 24-hour urinary Cr excretion was significantly influenced by both dietary protein and polyunsaturated fat intake.

Moreau, MC; Ducluzeau, R; Raibaud, P. (1976) **Hydrolysis of urea in the gastrointestinal tract of "monoxenic" rats: effect of immunization with strains of ureolytic bacteria**. Infect Immun 13: 9-15. Axenic rats, in whose feces urea is ordinarily excreted, were inoculated with ureolytic strains of Lactobacillus or Actinobacillus originally derived from the microflora of "holoxenic" rats. In these "monoxenic" animals, harboring one or another of the bacterial strains, fecal urea was hydrolyzed, with a more rapid onset of ureolysis in the case of Actinobacillus as compared with Lactobacillus. In vitro, a parallel difference between the two strains with regard to the onset of ureolysis was observed, hydrolysis beginning at the onset of growth in the case of Actinobacillus and only at the end of the exponential growth phase in the case of Lactobacillus. Extracellular urease activity was demonstrated in cultures of Lactobacillus, whereas none was found extracellularly with Actinobacillus. The pH optimum for the Lactobacillus urease in vitro was found to be 3.0, whereas the corresponding value for Actinobacillus was 6.0. In the two types of monoxenic rats, urea was consistently present in the small intestine and virtually absent from cecum and colon. Hydrolysis of urea in stomach was almost complete in rats bearing Lactobacillus but much less so in animals monoxenic with Actinobacillus, despite essentially equal numbers of organisms in that location. When rats carrying a monoflora of ureolytic Lactobacillus were immunized with either whole cells or soluble extract of the same organism, urea appeared in cecum and feces, indicating suppression of ureolytic activity. Immunization with an extract of nonureolytic Lactobacillus failed to produce such a result. Similar immunization techniques applied to animals monoassociated with ureolytic Actinobacillus did not alter ureolysis, and no appreciable quantity of urea appeared in feces. These studies demonstrate that it is indeed possible to inhibit the ureolytic activity of some bacteria in vivo by immunological means, but that the urease system of other organisms may not be as amenable to such manipulation.

Picou, D; Phillips, M. (1972) **A study with 15N-urea on the effects of a low protein diet and malnutrition on urea metabolism in children**. Clin Sci 43: 17P.

Tripathy, K; Klahr, S; Lotero, H. (1970) **Utilization of exogenous urea nitrogen in malnourished adults**. Metabolism 19: 253-262.

Wolfe, RR; Goodenough, RD; Wolfe, MH; et al. (1982) **Isotopic analysis of leucine and urea metabolism in exercising humans**. J Appl Physiol 52: 458-466. We have used the primed constant infusion of di-[15N]urea and [1-13C]leucine to determine the effects of mild exercise (approx 30% Vo2max for 105 min) on urea production and leucine metabolism in human subjects. The oxidation of plasma leucine was distinguished from the oxidation of leucine that never entered the plasma pool ("intracellular" leucine) by means of determining the enrichment of alpha-ketoisocaproic acid (alpha-KICA). Total leucine oxidation increased from 0.38 +/0 0.05 to 1.41 +/- 0.14 micromol . kg-1 . min-1 during exercise due to increases in the oxidation of plasma leucine (150%) and intracellular leucine (600%). Plasma leucine flux decreased slightly, but not significantly (0.1 greater than P greater than 0.05), and the percent of alpha-KICA derived from plasma leucine dropped significantly (P less than 0.05) from 79.5 +/- 4.3 at rest to 62.0 +/- 5.3% over the last 30 min of exercise. Despite the increase in leucine oxidation during exercise, urea concentration and production did not change. Thus in exercise urea production does not accurately reflect all aspects of amino acid metabolism.

Young, VR; El-Khoury, AE; Raguso, CA; et al. (2000) **Rates of urea production and hydrolysis and leucine oxidation change linearly over widely varying protein intakes in healthy adults**. J Nutr 130: 761-766. The quantitative relationships between nitrogen (N) intake, urea production, excretion and amino acid oxidation are currently a matter of debate. Some investigators have proposed that urea production is essentially constant over a wide range of N intakes and that urea hydrolysis is regulated according to the N needs of the organism. We have assessed this proposal by compiling results from four separate experiments in healthy young adults (n = 34) carried out in our laboratories and all at the end of the respective diet periods using an identical 24-h continuous intravenous infusion of [(15)N, (15)N] urea and L-[1-(13)C]leucine. The N intakes were: experiment 1; protein-free diet for 5 d; experiment 2; N at 44 mg N. kg(-1). d(-1) from a balanced L-amino acid mixture for 13 d; experiment 3; N at 161 mg. kg(-1). d(-1) from egg protein for 6 d; experiment 4 -one group received 157 mg. kg(-1). d(-1) and the other 392 mg. kg(-1). d(-1) from milk-protein-based diets for 6 d. Urea production and excretion were linearly correlated with N intake (r = 0.98 and 0.94, respectively; P < 0.01). Urea hydrolysis increased linearly with N intake (r = 0.7; P < 0.05), with considerable variation in the rate among individuals, especially at the N intake of approximately 160 mg N. kg(-1)d(-1). These findings are consistent with the generally accepted view that a control of body N balance is via a regulation of urea production. They do not support the concept that urea hydrolysis is the more important site in the control of body N loss.

3.4. ELIMINATION

Agarwal S C. (1955) **Some observations on urea-clearance test**. The Indian journal of medical research 43: 179-183.

Beza, R; Gruszczynska, J; Hacel, W; et al. (1993) **Studies on nitrogen metabolism in rats of different age fed on a protein-free diet. 4. Urinary urea nitrogen excretion in relation to body size and duration on protein-free diet feeding**. J Anim Feed Sci 2: 1-14. Excretion dynamics of urea nitrogen (NU) in endogenous urinary N (EUN) was studied in 350 male Wistar rats in 14 age groups, ranging

from 23 to 273 days old, with an average body weight (SB) of 39 to 446 g. Rats in each age group were randomly assigned to subgroups in which protein-free diet (PFD) was fed for 3, 6, 9, 12 or 16 days. Age of rats had a significantly greater influence on concentration of NU and EUN than duration of PFD feeding. Age and duration of PFD feeding interacted significantly. The proportion of NU in EUN increased as duration of PFD feeding increased up to about 80-100 days old, after which it increased only slightly in older rats. Proportion of NU in EUN decreased between 3 and 16 days of feeding PFD. NU excretion (mg) as a function of urinary EUN excretion (mg) and duration of PFD feeding was described by a linear multiple regression equation: NU = 0.27 x EUN1.187 x t-0.185, where t is duration of PFD feeding in days. Daily urea N excretion (NUd) was highly correlated with body weight (SBd), increasing with age and decreasing with duration of feeding PFD. This relation describes the multiple regression equation of which exponential transformation is: NUd = 225.4 x SBd1.033 x d-0.242 where SBd is expressed in kg and d is the day of PFD feeding. These equations allow estimation of the amount of endogenous NU in urine of rats fed for 16 days on PFD and NUd excretion on days 1 to 16 on PFD.

Balakhovskii, IS; Orlova, TA. (1983) **Mechanism of urea excretion after protein loading**. Hum Physiol 9: 200-204.

Baltzan, MA; Shoker, AA; Baltzan, RB; et al. (1996) **Improved urea clearance raises the BUN in continuous peritoneal dialysis**. Clin Nephrol 45: 183-187. Cross-sectional studies in steady state dialysed chronic end-stage renal failure patients show urea clearance (Kt/V) and total urea excretion (protein catabolic rate) correlate positively. However, urea clearance is total urea excretion divided by BUN. Thus urea clearance and BUN relate reciprocally, and so their mathematical product (total urea excretion) is independent of clearance. As such clearance cannot also be a positive correlate of total excretion as demanded by the cross-sectional studies. Furthermore the clearance formula dictates that the positive urea clearance and total urea excretion correlation found in the cross-sectional studies can only occur if the increased urea clearance fails to reciprocally lower the BUN. Thus the relations of urea clearance, urea excretion, and BUN requires further definition. To so define we examine dialysis urea excretion, dialysis urea clearance, BUN, and serum albumin in 13 stabilized chronic uremics with minimal native renal function who are treated by continuous ambulatory peritoneal dialysis (CAPD). Urea clearance and BUN correlate positively ($r = 0.62$, $p < 0.05$) and both also correlate positively with dialytic urea excretion and (urea clearance $r = 0.912$, $p < 0.001$, BUN $r = 0.88$, $p < 0.001$). In addition dialytic urea excretion and serum albumin indexed to body size correlate positively ($p < 0.05$). Thus in the steady state urea clearance associates with both an increase in BUN and urea output. However the law of conservation of mass makes urea output is a function of protein intake. Thus increased clearance cannot directly increase such output, and so increased clearance must first increase intake but in doing so it increases the retention of the byproducts of enhanced intake, BUN and other protein metabolites, so leading to a paradox, the more removed, the more remains. These observations taken together suggest that in chronic uremia treated by continuous dialysis, elevation of the BUN may be a marker for an adequate restoration of protein metabolism if inadequate dialysis is excluded.

Bankir, L; Bouby, N; Trinh-Trang-Tan, M M; et al. (1996) **Direct and indirect cost of urea excretion**. Kidney Int 49: 1598-1607. Urea, the major end product of protein metabolism in mammals, is the most abundant solute in the urine. Urea excretion is thought to result from filtration curtailed by some passive reabsorbtion along the nephron. This reabsorption is markedly enhanced by vasopressin and slow urinary flow rate (V), the fraction of filtered urea excreted in the urine (FEurea) falling from approximately 60% at high V to only approximately 20% at low V. In concentrated urine, normal urea excretion can be maintained only if urea filtration is elevated. This can be achieved by increasing plasma urea concentration (Purea) and/or GFR. We have shown that both parameters do increase when normal rats are submitted to chronic alterations in the water intake/vasopressin axis within the normal range of

physiologic regulation. This situation is very similar to that observed after alterations in protein intake. In both cases more urea needs to be filtered, either because more of it has to be excreted, or because the efficiency of its excretion is reduced. A common mechanism is proposed to explain the rise in GFR observed in the two situations. In summary, our studies demonstrate that the antidiuretic effects of vasopressin are responsible for a significant elevation of GFR. This GFR adaptation limits the rise in Purea, a favorable effect because urea is not as harmless as usually thought. However, this hyperfiltration might have deleterious consequences in diseased kidneys.

Bankir, L; Bouby, N; Yang, B. (2003) **Extremely high turnover of urea in mice compared to other mammals**. FASEB J 17: 4-5. Renal urea handling has been well documented in a several mammals but not in mice (M). Data collected in six wildtype CD1 male M was compared with previously published data obtained in Sprague Dawley rats (R) (Bouby et al, JASN. 7:842-851, 1996) in similar conditions except that R food contained 23 % casein and M food only 20 %. Twenty four h urine and a blood sample were collected, and urea and creatinine (Creat) were measured (Creat with an enzymatic method).) The two species exhibited a 10-fold difference in body weight (BW) (27.7 vs 260 g) but only a 4-fold difference in food intake. Creat clearance was 242 +- 11 ml/d in M and 2400 +- 59 in R. Plasma urea was 75 % higher in M than R (9.1 +- 0.1 vs 5.2 +- 0.5 mmol/L) and urine urea was more than double (1870 +- 110 vs 736 +- 84 mmol/L), resulting in a 50 % larger urine-to-plasma urea ratio, in agreement with the more efficient urinary concentrating capacity of the M kidney. Total body urea pool (calculated as plasma urea times extracellular fluid volume = 60 % BW) was only 5 times lower in M than in R (151 +- 10 vs 813 +- 115 mumol). Urea excretion amounted 3.74 +- 0.14 in M vs 7.53 +- 0.33 mmol/d in R, and urea fractional excretion (FE) was 175 +- 14 % in M vs 67 +- 7 % in R. Thus mice excrete each day an amount of urea equal to 25 times their urea pool vs only about 10 times in rats (and one time in humans, not shown). This extremely high excretion rate is possibly favored by an active urea secretion, as suggested by the high FE urea.

Beza, R; Gruszczynska, J; Hacel, W; et al. (1993) **Studies on nitrogen metabolism in rats of different age fed on a protein-free diet. 4. Urinary urea nitrogen excretion in relation to body size and duration on protein-free diet feeding**. J Anim Feed Sci 2: 1-14. Excretion dynamics of urea nitrogen (NU) in endogenous urinary N (EUN) was studied in 350 male Wistar rats in 14 age groups, ranging from 23 to 273 days old, with an average body weight (SB) of 39 to 446 g. Rats in each age group were randomly assigned to subgroups in which protein-free diet (PFD) was fed for 3, 6, 9, 12 or 16 days. Age of rats had a significantly greater influence on concentration of NU and EUN than duration of PFD feeding. Age and duration of PFD feeding interacted significantly. The proportion of NU in EUN increased as duration of PFD feeding increased up to about 80-100 days old, after which it increased only slightly in older rats. Proportion of NU in EUN decreased between 3 and 16 days of feeding PFD. NU excretion (mg) as a function of urinary EUN excretion (mg) and duration of PFD feeding was described by a linear multiple regression equation: NU = 0.27 x EUN1.187 x t-0.185, where t is duration of PFD feeding in days. Daily urea N excretion (NUd) was highly correlated with body weight (SBd), increasing with age and decreasing with duration of feeding PFD. This relation describes the multiple regression equation of which exponential transformation is: NUd = 225.4 x SBd1.033 x d-0.242 where SBd is expressed in kg and d is the day of PFD feeding. These equations allow estimation of the amount of endogenous NU in urine of rats fed for 16 days on PFD and NUd excretion on days 1 to 16 on PFD.

Bing, I; Bjering, T. (1938) **Individual variations in the relation between urea clearance and creatinin clearance**. Acta Med Scand Suppl 89: 64-65. There are considerable variations from the normal relationship (0.5) in patients with nephrosis (0.25-0.75). The "standard clearance" should only be employed when the concentrate index for urea is greater than 75. ABSTRACT AUTHORS: J. F. Wilkinson

Bingham, SA; Williams, R; Cole, TJ; et al. (1988) **Reference values for analytes of 24-h urine collections known to be complete**. Ann Clin Biochem 25: 610-619. One hundred and twenty two 24-h urine collections were obtained from a representative sample of men and women in the general population aged 25-44 years. The collections were verified for their completeness by urine recovery of oral doses of para-amino benzoic acid. Means (with 0.95 reference intervals) for both sexes for 24-h urinary output were determined for nitrogen, urea, creatinine, urate, ammonia, sodium, potassium, volume, and pH. Within person variation in nitrogen, urea, creatinine, ammonia, sodium and potassium was estimated for a consecutive 28-day series of 24-h urine collections for eight individuals. Within person variation was greatest for sodium and least for creatinine. These differences when compared with the between person variation in the analytes studied have implications for the precision with which a result from a single specimen can be used to place an individual within a distribution of values for epidemiological purposes. In clinical biochemistry, the population results reported here should be appropriate as a reference base for comparison with individual patient results.

Blackmore, DJ; Mason, JK. (1968) **Renal clearance of urea, creatinine and alcohol**. Med Sci Law 8: 50-53.

Brulles, A; Gras, J; Magrina, N; et al. (1969) **Relation between urea clearance and glomerular filtration rate according to urine flow-minute**. Clin Chim Acta 24: 261-265.

Brusilow, SW; Gordes, EH. (1965) **Secretion of urea and thiourea in sweat**. Am J Physiol 209: 1213-1218.

Chakravarti, MG; Mitra, S. (1951) **Micro estimations of nitrogenous constituents of the blood and of urea clearance; a study of 100 cases**. Ind Med Gaz 86: 343-348.

Chasis, H; Smith, HW. (1938) **The excretion of urea in normal man and in subjects with glomerulonephritis**. J Clin Invest 17: 347-358. The urea clearance was examined in 10 normal subjects and 22 subjects with glomerulonephritis, with special reference to the degree of concentrate of the glomerular filtrate as indicated by the simultaneous inulin U/P ratio. Urea is invariably reabsorbed to some extent from the glomerular filtrate, whether this is at the normal level or is reduced by disease. Water reabsorption occurs in 2 stages, one in the proximal tubule and one in the thin limb and distal tubule. At any inulin U/P ratio the reabsorption of urea proceeds in the nephritic kidney essentially as it would in the normal kidney. As the capacity to reabsorb water is impaired by disease. the fraction of urea reabsorbed decreases, so that the urea clearance approaches the rate of glomerular filtration. In none of the subjects examined was there evidence of increased back-diffusion of urea, the elevation of the blood urea in nephritis being a result solely of the reciprocal relationship between this term and the urea clearance, as expected in principle. ABSTRACT AUTHORS: Authors

De Santo, NG; Di Iorio, B; Capasso, G; et al. (1992) **Age-related changes of urinary urea. Epidemiological study in children from southern Italy living in Cimitile**. Child Nephrol Urol 12: 24-29. Urinary urea was measured, under normal living conditions, in children aged 3-16 years, from Cimitile, a small town near Naples in Southern Italy. Urinary urea correlated with age, height, weight, body mass index and body surface area, and was independent from sex. Urinary urea also correlated with urinary creatinine, uric acid, oxalate, sodium and phosphate. Protein intake assessed from urea excretion was 2.66 +/- 0.66 g/kg at 3 years of age and 1.49 +/- 0.31 g/kg at 16 years, and exceeded Italian standards for recommended intake. Also, the height and weight of the children living in Cimitile, with few exceptions, were in excess of national reference standards.

Farr, LE; Smadel, JE. (1936) **The urea clearance of rats: Its technique and normal range**. Am J Physiol 116: 349-357. A method is descr. for determining urea clearance of rat s. Urea clearances on normal rats, on a milk diet, determined in the manner described, showed a mean average of 10.9 cc. per sq. m. of body surface per min. The standard deviation was [plus or minus] 3.1 cc. ABSTRACT AUTHORS: L. E. Farr

Fenton, RA; Knepper, MA. (2007) **Urea and renal function in the 21st century: insights from knockout mice**. J Am Soc Nephrol 18: 679-688. Since the turn of the 21st century, gene knockout mice have been created for all major urea transporters that are expressed in the kidney: the collecting duct urea transporters UT-A1 and UT-A3, the descending thin limb isoform UT-A2, and the descending vasa recta isoform UT-B. This article discusses the new insights that the results from studies in these mice have produced in the understanding of the role of urea in the urinary concentrating mechanism and kidney function. Following is a summary of the major findings: (1) Urea accumulation in the inner medullary interstitium depends on rapid transport of urea from the inner medullary collecting duct (IMCD) lumen via UT-A1 and/or UT-A3; (2) as proposed by Robert Berliner and colleagues in the 1950s, the role of IMCD urea transporters in water conservation is to prevent a urea-induced osmotic diuresis; (3) the absence of IMCD urea transport does not prevent the concentration of NaCl in the inner medulla, contrary to what would be predicted from the passive countercurrent multiplier mechanism in the form proposed by Kokko and Rector and Stephenson; (4) deletion of UT-B (vasa recta isoform) has a much greater effect on urinary concentration than deletion of UT-A2 (descending limb isoform), suggesting that the recycling of urea between the vasa recta and the renal tubules quantitatively is less important than classic countercurrent exchange; and (5) urea reabsorption from the IMCD and the process of urea recycling are not important elements of the mechanism of protein-induced increases in GFR. In addition, the clinical relevance of these studies is discussed, and it is suggested that inhibitors that specifically target collecting duct urea transporters have the potential for clinical use as potassium-sparing diuretics that function by creation of urea-dependent osmotic diuresis.

Fowweather, FS. (1934) **Blood-urea clearance before and after giving urea**. Q J Med 3: 63-77. In 50 healthy [male] students the blood urea covered a wide range and in many fell below the normal fixed by van Slyke et al.; after urea ingestion the results occupied a narrow range, almost identical with that determined by van Slyke. These results and those of other exps. indicate that the blood urea clearance after urea alone should be used to determination renal function; also, that the 2d hr. after urea is less likely to convey error. Blood urea clearance was found to give a more correct indication of renal function than the urea concentration test.

Gordon, W; Alving, AS; Kretzschmar, NR; et al. (1937) **Variations in the extraction of urea by the kidney and their relation to the amount of urea reabsorbed**. Am J Physiol 119: 483-492. The following conclusions were based upon data from 50 exps. in one-kidney dogs in which the kidney was explanted by a modified Rhoads technique to insure collection of uncontaminated blood from the renal vein. Momentary variations in the extraction percentage of urea are dependent chiefly upon changes in the rate at which urea is reabsorbed, and to a relatively unimportant degree upon changes in the rate of filtration. The rate of reabsorption varies from none to all of the filtered urea and the average lies between 40 and 50%; this average is usually approximated within any period of 30-60 min. Cessation of the extraction of urea due to cessation of filtration can occur in association with anuria. Increase in urea concentration of the blood in the kidney does not exceed that explicable by complete reabsorption of filtered urea. It is postulated that extreme fluctuations in the rate of re- absorption of urea occur independently of experimental procedures. ABSTRACT AUTHORS: A. S. Alving

Holman, RL. (1933) **Observations on the urea clearance in dogs**. Am J Physiol 104: 615-623. A modification of the method of Moller, McIntosh and Van Slyke, using 24-hr. clearances instead of the usual 2-hr. tests, is suggested. Data on normal dogs indicate that fasting may reduce the urea clearance to 25% or less. Dogs with Eck fistulas, with biliary obstruction, and with liver injury due to chloroform anesthesia showed low blood urea levels, at the time of greatest liver injury, which were reflected in a moderate rise of urea clearance above control levels. ABSTRACT AUTHORS: R. L. Holman

Hubbard, RS; Griffith, FR. (1944) **The excretion of urea by normal subjects under basal conditions**. Am J Physiol 141: 469-475. The excretion of urea by two normal o subjects was determined under basal conditions over a period of a yr. 40 detns. were performed upon one and 43 upon the other subject. The data, which included the blood urea, the rate of urea excretion, the concentrate of urea in the urine, and various ratios between these factors, were submitted to statistical analysis. The blood concentrate and rate of urea excretion were quite constant for each subject, but the water excretion varied markedly. The urea concentrate in the blood was correlated with both the rate of excretion and the urea concentrate in the urine; the latter correlation was somewhat closer than the former. There was a slight negative correlation between the blood urea concentrate and the urine volume Although there was a slight positive correlation between the urine volume and the urea excretion, the general independence of the excretion of blood and urea was marked, and the urea excretion varied independently of changes in both the blood urea and the volume. The ratio between the rate of urea excretion and the blood urea was quite constant, and nearly the same for both subjects. ABSTRACT AUTHORS: R. S. Hubbard

Kleinman, LI; Radford, EP. (1963) **Studies of urea and inulin clearance in unanesthetized rats**. Fed Proc 22: 335 pp.

Litvinova, L; Viru, A; Smirnova, T. (1989) **Renal urea clearance in normal and adrenalectomized rats after exercise**. Jpn J Physiol 39: 713-723. In groups of male Wistar rats, blood level, urinary excretion of urea, and renal urea clearance were determined in resting conditions or during 48-60 h after swimming for 30 min with an additional load of 10% of body weight as well as after swimming for 3 or 10 h without additional load. Both types of swimming exercise caused a significant increase in the blood level, urinary excretion, and renal clearance of urea. After 10-h swimming, the increase of renal urea clearance was observed only from the second period (0-12 h) of urine collection. In this type of exercise, an elevation of blood level of corticosterone was obtained after 6-h restitution. The increased urinary excretion and renal urea clearance persisted for a longer time than was necessary for the normalization of the blood level. A significant rise in blood urea level and a drop in renal urea clearance occurred after adrenalectomy. In adrenalectomized rats, increased urea excretion and renal clearance were observed 12-24 h after 3-h swimming, but the level of renal clearance obtained in sedentary normal rats was not achieved. The blood level of urea did not increase. This suggested that the glucocorticoids play a role both in urea formation during exercise and in its elevated renal clearance after exercise.

MacKay, EM. (1932) **A comparison of the relation between the rate of urea excretion and the amount of renal tissue in the dog and other mammals**. Am J Physiol 100: 402-406. In 10 normal dogs adequate diuresis was provoked by fasting and administration of water and urea, the dog killed, the kidneys removed and the blood urea and urine urea estimated. The figures thus obtained were compared with those found by others for the rabbit and rat, and with figures indirectly obtained for man. In all 4 spp. the urine excretion ratio (urine urea rate:blood urea concentration) bore a direct relation to renal weight. In the dog the ratio value per gm. kidney weight is a great deal higher than for any of the other 3 spp. examined, in all of which the relationship is essentially the same. It is suggested that the dog's renal tubules may absorb less of the glomerular filtered urea than appears to be taken up by those of the frog,

or that while man, rabbit and rat are omnivorous or herbivorous, the dog (at least in the material used) is enitrely carnivorous, and protein temporarily increases the urea ratio:kidney weight relation.

Nice, M. (1935) **Kidney function during normal pregnancy. I. The increased urea clearance of normal pregnancy**. J Clin Invest 14: 575-578. The means for 93 antepartum and for 10 postpartum urea clearance tests, run in series on 13 normal women, were 153% and 105% respectively, with standard deviations of 45 and 12. These high clearances of normal pregnancy roughly paralleled their low blood ureas. ABSTRACT AUTHORS: M. Nice

Osswald, H; Auer, F. (1974) **Proceedings: Biliary elimination kinetics of tritium water, sodium-22, C-14 urea, C-14 creatinine, bromsulphthalein and H-3-ouabain injected into the portal vein of rats**. Naunyn Schmiedebergs Arch Pharmacol 282: suppl 282:R72.

Prior, RL; Milner, JA; Visek, WJ. (1975) **Urea, citrate and orotate excretion in growing rats fed amino acid-deficient diets**. J Nutr 105: 141-146. Male, weanling rats were fed a control purified amino acid diet or the same diet with lysine, phenylalanine, tryptophan, valine, or arginine omitted singly, or both arginine and lysine omitted. Blood urea reached three to four times that of control levels with all deficient diets. Urea excretion increased almost linearly with time during the first 3 days of amino acid deficiency. Rates of urea excretion on day 3 in decreasing order for various deficiencies were as follows: lysine and arginine combined are more than lysine is more than tryptophan equals valine is more that phenylalanine equals arginine. Urinary citrate was 26 and 21.8 times that of control values without arginine and 11.4 and 6.2 times that of control values without lysine on days 2 and 3, respectively. By day 8 citrate excretion had returned to control levels without lysine but not without arginine. Citrate excretion was unchanged with other deficiencies. Orotic acid excretion increased markedly only without arginine and slightly without tryptopahn. A deficiency of arginine and lysine increased urea and citrate excretions to a greater extent than either deficiency alone. Two injections of arginine or homoarginine (0.50 mmole/injection) given at 12-hour intervals to rats fed no lysine and arginine for 3 days decreased citrate excretion immediatedly and on the following day. Urea excretion decreased with injected homoarginine, but not with arginine. Orotic acid excretion increased more than four times on the day of homoarginine injection compared with that of the preceding day. Arginine injection returned orotic acid excretion to nearly control levels within 24 hours. Urea degradation in the gastrointestinal tract was increased in animals fed amino acid-deficient diets.

Rathaus, M; Bernheim, J. (1990) **Protein intake and 24-h urea excretion**. Nephrol Dial Transplant 5: 552-553.

Rotondi, AV. (1962) **Considerations on the influence of diuresis on urea clearance. Determination of the normal values of maximum urea clearance**. Rev Assoc Med Bras 8: 77-82.

Rudman, D; DiFulco, TJ; Galambos, JT; et al. (1973) **Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects**. J Clin Invest 52: 2241-2249. When normal individuals eat 0.33 g protein N/kg body weight (BW)((3/4)) per day, they excrete 10-15 mg urea N/h per kg BW((3/4)). If they now ingest (at 0 h) 0.27 (dose A), 0.40 (dose B), 0.53 (dose C), 0.94 (dose D), or 1.33 (dose E) g protein N/kg BW((3/4)) (in the form of casein, ovalbumin, or lactalbumin), the rate of urea N excretion accelerates within 4 h. At dose C a maximal rate of urinary urea N excretion (MRUE) is reached, which averages 55 mg urea N/h per kg BW((3/4)) and which persists for 16 h. Higher doses of protein do not further accelerate urea excretion, but prolong the duration of MRUE to 28 h (after dose E). Blood urea N (BUN) rises by 7-20 mg/100 ml during the first 8 h after dose C to E, and remains stable within +/-5 mg/100 ml during the ensuing 8-28 h of MRUE. Each increment of protein above dose C causes a further

increment in plasma alpha-amino N. During infusion of free amino acids at a rate of 110 or 165 mg amino acid N/h per kg BW((3/4)) for 12 h, rate of urea excretion increases to the MRUE value produced by dose C-E of oral protein. These findings indicate that MRUE corresponds to a period of maximal rate of urea synthesis (MRUS). MRUS is greater than MRUE because one fraction of newly formed urea is hydrolyzed in the gastrointestinal tract, and another fraction may accumulate temporarily in body water during the MRUE period. Oral neomycin reduces the proportion of urea hydrolyzed in the gut to less than 20%; its extent is measured by recovery in the urine of a tracer dose of [(14)C]urea injected intramuscularly during determination of MRUE. Accumulation of urea in body water is estimated from increment in BUN during the period of MRUE measurement (8-24 h after dose E of casein) and from body water measured with (3)H(2)O. Then MRUS is calculated as: ([mg urea N excreted between 8 and 24 h after dose E] + [BUN at 24 h - BUN at 8 h] x [body water]) x (100/% recovery [(14)C]urea) x (1/kg BW((3/4))) x (1/16 h).MRUS in 10 normal subjects averaged 65 mg urea N/h per kg BW((3/4)) (range 55-76), and in 34 cirrhotics 27 mg urea N/h per kg BW((3/4)) (range 6-64). Among 19 cirrhotic patients fed 40, 60, 80, or 100 g protein daily for successive 10 day periods, the occurrences of hyperammonemia, hyperaminoacidemia, and encephalopathy at each level of protein intake were inversely related to MRUS value.

Schmidt-Nielsen, B. (1958) **Urea excretion in mammals**. Physiol Rev 38: 139-168.

Schmidt-Nielsen, B. (1962) **Comparative physiology of urinary excretion**. Physiologist 5: 257-263.

Schmidt-Nielsen, B; Sands JA. (2001) **Urea excretion in white rats and kangaroo rats as influenced by excitement and by diet**. J Am Soc Nephrol 12: 856-864. Reprinted from The American Journal of Physiology, Vol. 181, No.1, April, 1955.

Shannon, JA. (1935) **Excretion of urea and creatinine in the dog in relation to rate of urine formation**. Proc Soc Exp Biol Med 33: 474-476. Preliminary paper.

Shannon, JA. (1936) **Glomerular filtration and urea excretion in relation to urine flow in the dog**. Am J Physiol 117: 206-225. The urea clearance in relation to urine flow was examined under a variety of conditions in 6 dogs (824 clearance periods), the simultaneous creatinine clearance being used in the majority of the periods in 5 dogs to measure the rate of glomerular filtration. The rate of a glomerular filtration is essentially constant and unrelated to the rate of urine flow in the ordinary exptl. range of the latter. It may, however, be depressed by dehydration, or elevated by the administration of large doses of water. At the highest urine flow obtainable (creatinine U/P ratio = 10) about 40% of the urea filtered is reabsorbed. As the urine flow decreases an increased fraction of the filtered urea is reabsorbed; the increase in the reabsorbed fraction being approximately related to the logarithm of the creatinine U/P ratio. Diuresis following a low urine flow is accompanied by a marked, transient exaltation of the urea clearance relative to the creatinine clearance, which may disappear before the peak of diuresis is reached. This exaltation of urea clearance is evoked by osmotic diuresis in a normal or pituitrinized dog, as well as by water diuresis in the normal. The concept of an augmentation limit with its corollary of standard and maximum clearance does not appear to be applicable to excretion in the dog. A simple diffusion hypothesis, positing that urea escapes from the tubule distal to the point of water reabsorption and in consequence of the concentration gradient created by this reabsorption is alone inadequate to explain the observed relationships between the deficit in the urea clearance and the rate of urine formation. ABSTRACT AUTHORS: J. A. Shannon

Stanier, MW. (1969) **The effect of urea loading on volume and concentration of urine in rabbits**. J Physiol 205: 367-376. 1. To find how urea contributes to the water-conserving ability of a herbivore's

kidney, groups of ten young rabbits on a low-protein diet and at three different levels of dietary electrolyte were given 1.8 g urea by mouth daily for 3 days. Vasopressin was administered daily to half the animals in each group.2. The urinary osmolarity and urea output of each animal was recorded daily during the urea loading and for a 3-day control period before and after loading. The renal water requirement for non-urea solute output (defined as daily volume/daily non-urea solute output) was calculated. The sodium content of renal cortex and medulla was measured in some animals from each group.3. Urea caused additional water excretion only in those rabbits which were receiving the low-salt diet. There was invariably increased water excretion when the ratio of urea to non-urea solute output exceeded 2.4. In most of the rabbits on normal-salt and high-salt intake, urea produced little change in the volume in which non-urea solute was excreted. Three out of the ten high-salt animals showed significant reduction of this volume during urea-loading.5. Vasopressin significantly reduced the volume required for non-urea solute output, but the effect of vasopressin was independent of urea-loading and of dietary electrolyte level.6. The low-electrolyte diet significantly reduced the sodium concentration in the rabbits' renal medullary tissue.7. It is concluded that in rabbits urea contributes to water retention mainly by its high permeability, enhanced by vasopressin, which permits maximal water reabsorption in the renal medulla. Water retention by means of uphill transport of urea, if it occurs at all, is slight.

Steffee, WP; Anderson, CF; Young, VR. (1981) **An evaluation of the diurnal rhythm of urea excretion in healthy young adults**. JPEN J Parenter Enteral Nutr 5: 378-384. A diurnal variation in urine urea excretion has been documented to exist in healthy young adults. Its persistence when dietary nitrogen intake approaches zero suggests that influences other than diet may in part generate the rhythm. These may include variations in the efficiency of rates of endogenous protein turnover of perhaps changes in renal tubular handling of urea especially in states of dietary nitrogen inadequacy. Recognition of these effects in the normal state are important before ascribing importance to changes in the disease state, particularly as we gain the ability to intervene nutritionally in the hospital setting.

Szanyiova, M; Leng, L; Faix, S. (1993) **Effect of 2 inhibitors of oxidative phosphorylation on urea excretion in rats**. Vet Res 24: 305-310. The effects of phloretin and carbonyl-cyanide-m-chlorophenylhydrazone (CCCP), both inhibitors of oxidative phosphorylation, on renal urea excretion in Wistar rats were investigated. Phloretin and CCCP infusions did not influence plasma urea concentration (P(urea)), compared with controls (0.15 M NaCl and Tris solution in 0.15 M NaCl-a solvent for phloretin and CCCP). The fractional urea excretion (FEurea) was not altered by phloretin infusion. It decreased significantly only when compared with 0.15 M NaCl infusion ($P < 0.05$). CCCP infusion had no effect on FEurea. The total amount of urea excreted by urine (UureaV) was not altered by phloretin compared with controls. CCCP significantly enhanced Uurea V only when compared with 0.15 M NaCl ($P < 0.001$), not when compared with Tris. Glomerular Filtration rate (GFR) increased significantly during phloretin infusion ($P < 0.001$), CCCP ($P < 0.001$) and also after Tris in 0.15 M NaCl ($P < 0.001$), in comparison with 0.15 M NaCl alone. Our results showed that phloretin and CCCP had no effect on urea excretion in rats. The increase in GFR is attributed to Tris, not to phloretin or CCCP. It is concluded that inhibition of oxidative phosphorylation in kidney has no effect on urea excretion.

Van Slyke, DD; Page, IH; Hiller, A; et al. (1935) **Studies of urea excretion 9. Comparison of urea clearances calculated from the excretion of urea, of urea plus ammonia, and of nitrogen determinable by hypobromite**. J Clin Invest 14: 901-910. Expts. with human subjects show that when the proportion of urea in the urea + ammonia mixture of the urine is markedly decreased by induced acidosis and low protein diet, the urea clearances calculated from the excretion rate of urea alone suffer a parallel reduction. If, however, values for excretion of urea + ammonia are substituted for urea, the clearances calculated remain at the usual levels. The theoretical significance of the results is to favor the hypotheses, that the ammonia excreted in the urine of man is formed in the kidneys chiefly from urea

removed from the blood, and that the work of the kidneys in excreting urea from the blood is more accurately indicated by the combined excretion of urea and ammonia than by the excretion of urea alone. The practical deduction is that in determinations of the urea clearance as a measure of renal function, results are somewhat more consistent if in the clearance formula, UV/B or U(Vi)/B, one uses for U the urinary concentration of urea + ammonia N, instead of only urea N. When the urinary urea is determined by methods measuring the NH3 formed by urea hydrolysis, or the N2 yielded by the action of hypobromite, the determination of combined urea + ammonia N is also simpler than determining the urea N separately. The routine procedure developed for clinical determination of the urea clearance is described. ABSTRACT AUTHORS: D. D. Van Slyke

Vasilyeva, VF. (1957) **Excretion of urea from high blood concentrations**. Biull Eksp Biol Med 44: 1443-1446. The value of filtration by inulin method (Cin) and urea clearance (Cu) in its increased concentration in the blood were determined simultaneously. It was established that Cu/Cin ratio increases with reduction of concentration index of inulin caused by the loading of urea. The Cu/Cin ratio almost equals 1, but is never over it. Concentration of urea in the blood does not stimulate its excretion by the tubular epithelium. ABSTRACT AUTHORS: Author

Walser, M; Bodenlos, LJ. (1959) **Urea metabolism in man**. J Clin Invest 38: 1617-1626.

Watanabe, J; Hirate, J; Iwamoto, K; et al. (1981) **Disposition of creatinine and urea in bilaterally nephrectomized rats**. J Pharmacobiodyn 4: 596-603. Bilaterally nephrectomized rats were used to investigate the disposition of creatinine when renal function is acutely decreased. The percentage of radioactivity recovered in the expired air in 11 hours following intravenous administration of [carbonyl-14C] creatinine immediately and 24 hours after nephrectomy was 0.54 and 3.24% respectively, and these values were significantly different (p less than 0.05). As it was considered that expiratory excretion is one of the major elimination routes in nephrectomized rats, mechanism of expiratory excretion was investigated by incubating [carbonyl-14C] creatinine with the intestinal contents of the rats chronically loaded with creatinine [carbonyl-14C] Creatinine was completely metabolized in that intestinal contents, but 14CO2 was not produced. When the mixture of the metabolites was administered orally or intravenously to chronically creatinine loaded rats, however, about 50% of the total radioactivity was excreted into the expired air in 5 hours. Furthermore, biliary excretion of the radioactivity following intravenous administration of [carbonyl-14] creatinine in the nephrectomized rats was much greater than that in normal rats. These results indicate that expiratory excretion following intravenous administration [carbonyl-14C] creatinine to nephrectomized rats would arise following intestinal absorption of the creatinine metabolites, which seem to be produced by intestinal microflora after biliary excretion of creatinine. The change of disposition for urea by bilaterally nephrectomy was also studied to compare with that for creatinine.

Watanabe, J; Mizuno, S; Masuda, N; et al. (1984) **Salivary excretion of urea in dogs**. J Pharmacobiodyn 7: 294-303. The salivary excretion of urea was investigated by collecting parotid saliva (Pr) and mandibular-sublingual saliva (MS) separately in beagle dogs. (1) After intravenous administration of urea (1.5 g/kg), urea concentrations in both Pr and MS were well correlated to but were lower than those in plasma. Urea concentrations in MS were significantly lower than those in Pr (p less than 0.05), indicating that there was glandular difference in salivary excretion of urea. (2) This glandular difference was not explained by Matin's equation, even if all variation factors in this equation were considered. (3) At relatively low salivary flow rates, the increase in saliva/plasma urea concentration ratio (S/P ratio) for both Pr and MS was found in the experiments for endogenous urea. (4) At relatively low salivary flow rates, glandular difference was observed in the S/P ratios of sodium ion. (5) Salivary

clearance of urea was highly dependent on salivary flow rate under stimulated condition for salivation, and mean value of the salivary clearance of urea was about 20% of its total body clearance.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Ando, A; Orita, Y; Shirai, D; et al. (1968) **Studies on renal function test report 57 mathematical analysis of urea clearance**. Jpn Circ J 32: 680-681.

Bourne, GR; Barber, HE. (1972) **The pharmacokinetics of inulin and urea: a comparison of the dose eliminated from a compartmental model and that eliminated in urine**. J Pharm Pharmacol 24: 532-537.

Campollo, O; Nikolaou, N; Carson, E; et al. (1989) **Mathematical modelling of urea kinetics a large scale compartmental model and a compact model**. Hepatology 10: 28-31.

Daugirdas, JT; Smye, SW. (1997) **Effect of a two compartment distribution on apparent urea distribution volume**. Kidney Int 51: 1270-1273.

El-Khoury, AE; Borgonha, S; Pereira, PCM; et al. (1999) **24-Hour leucine and urea kinetics at a low protein intake in healthy young men**. FASEB J 13: 17-21.

Forslund, A; El-Khoury, AE; Olsson, R; et al. (1995) **Effect of moderate physical activity upon protein and energy homeostasis in healthy adult humans: 24H studies with leucine and urea stable isotope tracers**. FASEB J 9: 9-13.

Forslund, AH; Hambraeus, L; El-Khoury, AE; et al. (1998) **24h Studies on whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults**. FASEB J 12: 18-22.

Forslund, AH; Hambraeus, L; Olsson, RM; et al. (1998) **The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults**. Am J Physiol 275: E310-320.

Gotch, FA. (1995) **Urea kinetic modelling**. Nephrol Dial 10: 2378-2379.

Gotch, FA. (2001) **Evolution of the single-pool urea kinetic model**. Semin Dial 14: 252-256. Our interest in urea kinetic modeling (UKM) was stimulated some 30 years ago at the time of the advent of hollow fiber kidneys with greatly improved urea transport. This led to examination of the interaction between time and clearance in computing the dialysis dose. In early studies a fixed-volume single-pool UKM was used but this frequently gave spurious high volumes and led to the advent of the variable-volume single-pool model. The role of volume calculation in assessment of the delivered dialysis dose and the value of normalized protein catabolic rate (nPCR) calculation are reviewed. More recently quantification of double-pool effects has become simplified and now is widely used for UKM. The National Cooperative Dialysis Study (NCDS) resulted in the concept of dose quantification by Kt/V. This is reviewed, including the controversy surrounding interpretation of the NCDS. Currently there is great interest in more frequent dialysis, 4-6 days/week. The development of a new dose parameter, the standard Kt/V (stdKt/V), to enable quantitative comparison of dose with widely varying dose schedules is discussed.

Juillet, B; Fouillet, H; Bos, C; et al. (2005) **Modulation of the dietary nitrogen metabolic fate and urea recycling by the nature of protein in humans: a compartmental analysis**. FASEB J 19: A1001. A 13-compartment model was developed to simulate the postprandial dietary nitrogen IN) distribution and metabolism in humans. This model assessed intestinalurea recycling that has been suggested to constitute a nitrogen sparing mechanism but has still been scarcely studied for methodological reasons. The modelwas built and validated from experimental data obtained in subjects having ingested a single liquid, mixed meal containing N-15-labeled milk or soy protein, after a one week-adaptation to a rich 2 g.kg(-1).d(-1) protein diet. Dietary N kinetics were measured in plasma proteins and amino acids, body urea, urinary urea and ammonia during 8h after the meal ingestion. The model enabled the simulation of the dietary N absorption, elimination and distribution in the splanchnic and peripheral areas. The results showed a faster gastric emptying of dietary N after soy vs. milk protein ingestion (half-time: 79 and 104 min, respectively), which was associated with its concurrent 20% higher transfer to area and its transiently greater anabolic utilization in the splanchnic bed, leading to a 20%-lower uptake and anabolic utilization of dietary N by the peripheral tissues. Furthermore, dietary N recycling from urea was greater after soy vs milk, with 11% and 5% of the urea N produced from dietary N reincorporated into splanchnic amino acids at 6h, respectively.This may allow a N-sparing mechanism counterbalancing the higher deamination of soy protein. Such a model thus constitutes a useful, explanatory tool to describe the processes involved in the metabolic utilization of various dietary proteins in humans.

Kaplan, B; Wang, Z; Siddhom, O; et al. (1999) **Evaluation of urea kinetics utilizing stable isotope urea and pharmacokinetic modeling**. Artif Organs 23: 44-50. The determination of urea kinetics plays a central role in clinical dialysis prescription. There persist, however, significant limitations to current approaches, particularly as they pertain to rigorous explorations of urea metabolism, distribution, and removal. This report describes methodologies designed to address these limitations by coupling a stable nitrogen isotope method with strict compartmental pharmacokinetic modeling. The findings of the present study can be summarized as follows. First, the use of stable isotope labeled exogenous urea is a reliable clinically applicable method for determination of urea kinetics. Second, this method offers significant advantages in that it allows for an accurate measurement of urea distribution space, endogenous urea production, and non-renal clearance of urea. Third, this method is significantly more rigorous than urea kinetic models that utilize only endogenous urea and do not carefully fit data points. Finally, pharmacokinetic modeling suggests that a two-compartment model satisfies all aspects of urea distribution and removal, but these compartments should not be equated with specific physiologic spaces. The combination of stable isotope urea compartmental modeling is a rigorous methodology for the assessment and validation of urea kinetics.

Kloppenburg, WD; Wolthers, BG; Stellaard, F; et al. (1997) **Determination of urea kinetics by isotope dilution with [13C]urea and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) analysis**. Clin Sci 93: 73-80. 1. Stable urea isotopes can be used to study urea kinetics in humans. The use of stable urea isotopes for studying urea kinetic parameters in humans on a large scale is hampered by the high costs of the labelled material. We devised a urea dilution for measurement of the distribution volume, production rate and clearance of urea in healthy subjects and renal failure patients using the inexpensive single labelled [13C]urea isotope with subsequent analysis by headspace chromatography-isotope ratio MS (GC-IRMS) of the [13C]urea enrichment. 2. The method involves measurement of the molar percentage excess of [13C]urea in plasma samples taken over a 4 h period after an intravenous bolus injection of [13C]urea. During the sample processing procedure, the plasma samples together with calibration samples containing a known molar percentage excess of [13C]urea are acidified with phosphoric acid to remove endogenous CO2, and are subsequently incubated with urease to convert the urea present in the plasma samples into CO2. The 13C enrichment of the generated CO2 is analysed by means of GC-IRMS. This method allows measurement of the molar percentage excess of [13C]urea to an

accuracy of 0.02%. 3. Reproducibility studies showed that the sample processing procedure [within-run coefficient of variation (CV) < 2.8% and between-run CV < 8.8%] and the GC-IRMS analysis (within-day CV < 1.3% and between-day CV < 1.3%) could be repeated with good reproducibility. 4. In clinical urea kinetic studies in a healthy subject and in a renal failure patient without residual renal function, reproducible values of the distribution volume, production rate and clearance of urea were determined using minimal amounts of [13C] urea (25-50 mg). 5. Because only low [13C]urea enrichments are needed in this urea dilution method using GC-IRMS analysis, the costs of urea kinetic studies are reduced considerably, especially in patients with renal failure.

Lifson, N; Hakim, AA. (1966) **Simple diffusive-convective model for intestinal absorption of a nonelectrolyte (urea)**. Am J Physiol 211: 1137-1146.

Marini, JC; Lee, B; Garlick, PJ. (2006) **In vivo urea kinetic studies in conscious mice**. J Nutr 136: 202-206. Stable isotope studies in conscious mice have been limited by the invasive catheterization procedures and relatively large sample size required. We developed minimally invasive catheterization protocols that together with the ability to analyze small samples have allowed for the study of urea kinetics in conscious mice. A single dose of 15N15N-urea followed by multiple sampling in mice (n = 6) showed that a primary pool of urea exchanged rapidly [70.65 +/- 14.96 mmol/(kg x h)] with a secondary pool. The urea entry rate determined with this protocol was 3.36 +/- 0.30 mmol/(kg x h). Continuous infusion of 15N15N-urea (n = 6) achieved plateau enrichment values at 3.3 +/- 0.2.h from which the urea entry rate was determined by isotope dilution [3.24 +/- 0.23 mmol/(kg x h)]. The urea entry rate measured by the single dose or continuous infusion protocol did not differ (P = 0.76). The minimally invasive methods described allow us to study not only ureagenesis and urea cycle disorders in vivo, but also urea transport and transporter function and nitrogen metabolism in general in mouse models. This is especially relevant because mouse targeting technologies will likely facilitate the generation of organ and tissue specific nulls of the various urea cycle enzymes.

Misra, M; Nolph, K. (2006) **A simplified approach to understanding urea kinetics in peritoneal dialysis and hemodialysis**. Contrib Nephrol 150: 20-27. Urea, a small molecular solute, is the candidate molecule commonly used to understand solute kinetics both in peritoneal dialysis and hemodialysis. Serum urea or serum urea nitrogen levels are utilized to calculate measures of dialysis adequacy and nutritional status in dialysis patients. The kinetic behavior of this molecule is however different for a continuous therapy like peritoneal dialysis vis a vis an intermittent therapy like hemodialysis. This article presents a simplified approach to understanding urea kinetics on dialysis.

Odeh, YK; Wang, Z; Ruo, TI; et al. (1993) **Simultaneous analysis of inulin and 15N2-urea kinetics in humans**. Clin Pharmacol Ther 53: 419-425. To elucidate the physiologic basis of multicompartmental systems used to model drug distribution, we studied inulin and 15N2-urea kinetics after simultaneous intravenous injection in five normal subjects. Distribution of both compounds was characterized by three-compartment models in which the central compartment corresponded to intravascular space. The mean distribution volumes of 0.164 +/- 0.009 L/kg (+/- SD) for inulin and of 0.670 +/- 0.143 L/kg for urea were similar to expected values for extracellular space and total body water, respectively. Distribution from intravascular space was kinetically heterogeneous, presumably reflecting differences in vascular beds supplied by either fenestrated and discontinuous capillaries or capillaries with a continuous basement membrane. Intercompartmental clearances of inulin and urea and the ratio of their free water diffusion coefficients were used to estimate blood flows and permeability coefficient-surface area products for the peripheral compartments. The sum of compartmental blood flows averaged 5.39 +/- 0.49 L/min and was similar to dual-beam Doppler measurements of cardiac output (5.47 +/- 0.40 L/min).

Pearson, P; Lew, S; Abramson, F; et al. (1994) **Measurement of kinetic parameters for urea in end-stage renal disease patients using a two-compartment model**. J Am Soc Nephrol 4: 1869-1873. Urea kinetic modeling depends critically on the parameters of the model used. When urea is removed during hemodialysis, the kinetic model is quite complex. This experiment describes for the first time the use of injected stable isotope-labeled urea to define kinetics in ESRD patients and compares the magnitude of the two urea compartments in these patients with those of control subjects. Such an experimental approach provides the kinetic data in the most direct manner. A gas chromatograph/mass spectrometer-based assay provided quantitation to as little as 0.2 mol% excess urea. The rapidly equilibrating fraction of the two urea compartments is quantified as 41.2% in ESRD patients and 33.4% in controls (P = 0.24). The rest is in a more slowly equilibrating pool. The urea clearances between these two compartments were near 1 L/min for both sets of subjects. The elimination of urea was due to the metabolic removal of the 15N label in both groups of subjects as well as renal elimination in the controls. The nearly threefold larger clearance (Cl) of labeled urea removal in controls (Cl = 74.6 mL/min) than in ESRD patients (Cl = 25.4 mL/min; P = 0.015) shows the extent to which renal clearance is more important than metabolism. These direct analyses of the fractional volumes and intercompartmental clearances for urea agree closely with previous measurements during high-efficiency hemodialysis and indicate that ESRD patients do not differ significantly from control subjects in these parameters.

Sedek, GS; Ruo, TI; Frederiksen, MC; et al. (1989) **Splanchnic tissues are a major part of the rapid distribution spaces of inulin, urea and theophylline**. J Pharmacol Exp Ther 251: 1026-1031. Distribution of kinetics of inulin, [14C]urea and theophylline were studied in five anesthetized dogs after splenectomy and gastrointestinal resection. Distribution was modeled with three-compartment mammillary systems in which the central compartment corresponds to intravascular space and the two peripheral compartments have different rates of transcapillary exchange. Compared with results in intact dogs, the surgical procedure removed between 41 and 55% of the rapidly equilibrating tissues and reduced the permeability coefficient-surface area products for the rapidly equilibrating inulin and urea compartments proportionately. This is consistent with the concept that splanchnic organs equilibrate rapidly with inulin and urea because they are supplied by fenestrated and discontinuous capillaries that are prominent in the splanchnic vascular bed. However, splanchnic organs probably do not contain all rapidly equilibrating tissues, and somatic tissues may contribute as much as 36 and 22%, respectively, of the rapidly equilibrating inulin and urea compartments. Cardiac output averaged 2.87 +/- 0.86 liters/min and was similar to the sum of compartmental blood flows estimated from the intercompartmental clearances of urea and inulin (2.74 +/- 0.96 liters/min) and to the sum of theophylline intercompartmental clearances (2.62 +/- 0.74 liters/min). Theophylline intercompartmental clearance to each peripheral compartment was similar to estimated compartmental blood flow.

Vanholder, R; Van Trimpont, P; Ringoir, S. (1989) **Urea kinetic modelling: comparison of three methods**. J Med Eng Technol 13: 87-89. It has been claimed that computed urea kinetic (UK) modelling in haemodialysed patients for the estimation of protein intake and of the relation between total dialyser urea clearance and distribution volume (Kt/V) leads to an overestimation of protein catabolic rate (PCR). In the present study three different methods of kinetic modelling for the determination of PCR and Kt/V are compared in 15 patients. The first method (MI) is the direct quantification method based on the collection of all urea eliminated from the body. The two other methods are based on an iterative computed calculation. The second method (MII) is the urea kinetic modelling method as described by Sargent. Dialyser clearances were measured directly and not estimated by theoretical extrapolation. The third method described here (MIII), is based on the indirect calculation of urea distribution volume (Vw) according to Watson and of dialyser clearances from this Vw and from pre- and post-dialysis urea concentrations. All three methods result in PCRs that are not significantly different (MI: 1.04 +/- 0.29; MII: 1.07 +/- 0.28; MIII: 1.05 +/- 0.24 mg/kg BW per 24 h; p greater than 0.05). When the results are

correlated, the following results are obtained: MI vs MII: r = 0.76, p less than 0.001; MI vs MIII: r = 0.78, p less than 0.001; MII vs MIII: r = 0.90, p less than 0.001. For Kt/V virtually identical results were obtained for each of the methods under study. In conclusion, all methods under study seem equally reliable in determining mean PCR.(ABSTRACT TRUNCATED AT 250 WORDS)

Vanholder, R; Van Trimpont, P; Ringoir, S. (1990) **Comparison of two methods for the estimation of urea kinetics and introduction of a third simplified method**. Int J Artif Organs 13: 663-666. It has been claimed that computed urea kinetic (UK) modelling in hemodialysed patients, for the estimation of protein intake, leads to an overestimation of protein catabolic rate (PCR). In the present study, three different methods of kinetic modelling for the determination of PCR and Kt/V are compared in 24 patients. The first method was the direct quantification method (DDQ) based on the collection of all urea eliminated from the body. The first computed method (ICMI) was the urea kinetic modelling method as described by Sargent. Dialyzer clearances were measured directly and not estimated by theoretical extrapolation. The second computed method (ICMII) is based on the indirect calculation of urea distribution volume (Vu), according to Watson, and of dialyzer clearances from this Vu and from pre- and post-dialysis urea concentrations. All three methods resulted in PCR's that were not significantly different (DDQ: 1.03 +/- 0.19; ICMI: 1.04 +/- 0.22; ICMII: 1.08 +/- 0.25 mg/Kg BW.24 hrs; p greater than 0.05). When the results were correlated, the following results were obtained: ICMI vs ICMII: r = 0.89, p less than 0.001; ICMI vs DDQ: r = 0.68, p less than 0.01; DDQ vs ICMII: r = 0.78, p less than 0.001. Intermutual comparison of Kt/V values resulted in virtually identical results, especially when comparing ICMI and ICMII, where the regression line equalled the identity line. In conclusion, all methods seem equally reliable in determining mean PCR and Kt/V. Our data, obtained with directly measured dialyzer urea clearances, do not confirm the earlier held opinion that computed modelling results in an overestimation of PCR.(ABSTRACT TRUNCATED AT 250 WORDS)

Wesson, LG. (1954) **A theoretical analysis of urea excretion by the mammalian kidney**. Am J Physiol 179: 364-371. Equations, based upon the hypothesis that the nephron comprises 2 regions of distinct but different permeability to urea, are derived which describe urea clearance in terms of glomerular filtration rate, regional permeabilities and urine flow. Calculation of permeability constants and behavior of the equations under various conditions are illustrated. The equations predict the changes in urea/inulin clearance ratios attendant upon water and osmotic diuresis and upon filtration rate changes. Agreement with available experimental data is good. ABSTRACT AUTHORS: L. G. Wesson, Jr

4. HAZARD IDENTIFICATION

4.1 STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Aagaard NK; Thogersen T; Grofte T; et al. (2004) **Alcohol acutely down-regulates urea synthesis in normal men**. Alcohol Clin Exp Res 28: 697-701. BACKGROUND: Human nitrogen balance studies suggest that alcohol up-regulates urea synthesis and promotes nitrogen catabolism, whereas animal studies conversely indicate that alcohol down-regulates urea synthesis, possibly via a redox effect. This study aimed to investigate the acute effects of alcohol exposure at a plasma concentration of about 10 mmol/liter on urea synthesis in healthy volunteers and to investigate whether methylene blue alleviates the effect of alcohol. METHODS: Eleven males were studied three times in a randomized sequence crossover design. They received either alanine infusion to control the rate of urea synthesis (control), alanine + alcohol, or alanine + alcohol + methylene blue. The substrate independent regulation of urea synthesis was studied by means of the functional hepatic nitrogen clearance, that is, the slope of the linear relation between blood amino nitrogen concentrations and rates of urea synthesis. RESULTS: Alcohol reduced functional hepatic nitrogen clearance to 37% and 51% during alcohol and alcohol + methylene blue infusion, respectively ($p = 0.007$). Accordingly, whole body nitrogen retention was higher during alcohol infusion. Glucagon, which up-regulates urea synthesis, increased during alcohol infusion. There was no change in insulin. Blood glucose was slightly lower at the end of the experiment when alcohol was infused. CONCLUSION: Low-dose infusion of alcohol acutely down-regulated urea synthesis in healthy volunteers, transiently favoring nitrogen preservation. The effect seemed not to depend on hormonal changes. It remains to be explored how the present results can be reconciled with the reported nitrogen wasting of chronic alcoholics.

Agner T. (1992) **An experimental study of irritant effects of urea in different vehicles**. Acta Derm Venereol Suppl (Stockh) 177: 44-46. The properties of urea as an irritant were investigated. Seventeen healthy volunteers were patch tested with 20% urea using petrolatum and water, respectively, as vehicles. Irritant effects of urea were assessed by clinical evaluation of patch test reactions as well as by various non-invasive methods. The inflammatory response was quantified by laser Doppler flowmetry measuring the superficial blood flow, and by ultrasound A-scan reflecting the edema formation. Impairment of the barrier function was indicated by measurement of transepidermal water loss (TEWL). It is concluded that 20% urea in petrolatum applied under occlusion for 24 h elicits significant inflammation (i.e. increase in blood flow and skin thickness) and causes impairment of the skin barrier (i.e. increased TEWL). The irritant impact of urea on the skin depends upon the vehicle used, the irritant effect being intensified when urea is dispensed in petrolatum compared with water.

Arguelles B; Barja J; Hernandez SMR; et al. (1994) **[Reference values of urea, creatinine, and creatinine clearance in children and adolescents]. VALORES DE REFERENCIA DE UREA, CREATININA Y ACLARAMIENTO DE CREATININA EN NINOS Y ADOLESCENTES**. Nefrologia 14: 175-180. We have studied 2,030 children and adolescents between 3 and 18 years of age (578 girls and 622 boys). We obtained reference values of creatinine, urea and urea/creatinine serum ratio in all of them, and creatinine clearance (Cl(cr)) in 1,200. We found an increase in serum creatinine levels with age in both sexes, with an uncharging serum urea level that results in a decrease of serum urea /creatinine ratio. The Cl(cr) increase with age, but not with sex. We suggest the use of the formula: FG = K.L/Pcr to determinate renal function, it was derived from comparison with Cl(cr) in 10 h. urine collections.

Arrazola P; Abramovits W. (2005) **ZODERM (benzoyl peroxide with urea in cream, gel, and cleanser preparations)**. Skinmed 4: 42-43.

Ashton H; Frenk E; Stevenson CJ. (1971) **Therapeutics 13. Urea as a topical agent**. Br J Dermatol 84: 194-196.

Bensinger TA; Mahmood L; Conrad ME; et al. (1972) **The effect of oral urea administration on red cell survival in sickle cell disease**. Am J Med Sci 264: 283-287.

Berger M; Standl E. (1981) **[Sulfonyl urea compounds in diabetes therapy 1981. Arguments for a reasonable use]**. Dtsch Med Wochenschr 106: 1443-1446.

Bhat, MR; Ramaswamy, C. (1993) **Effect of ammonia, urea and diammonium phosphate (DAP) on lung functions in fertilizer plant workers**. Indian J Physiol Pharmacol 37: 221-224. A spirometric study (FVC, FEV1 & PEFR/Min) was carried out in workers exposed to fertilizer chemicals (91) as a whole, out of which 31 were Ammonia, 30 Urea and 30 DAP plant workers, and 68 were controls belonging to the same age, sex, body surface area and socio-economic status. This study showed significant reduction in both PEFR/min and FEV1, the reduction being more in PEFR/Min., seems to indicate obstructive type of lung changes affecting the larger air-ways first, followed by bronchospasm. But FVC showed a significant reduction only after more than 10 years of exposure, probably causing restrictive changes only after prolonged exposure. Out of the 3 chemicals, DAP affected all the 3 parameters most, followed by Ammonia and Urea affected the least.

Blumenfeld TA; Mantell CH; Catherman RL; et al. (1978) **Post mortem vitreous concentrations of sodium potassium chloride calcium magnesium ions urea nitrogen creatinine and total protein in children dying of sudden infant death syndrome and known causes**. Am J Clin Pathol 70: 0002-9173.

Bonnet M. (1967) **[Pupillary blockage after urea perfusion in a case of aphakia]**. Bull Soc Ophtalmol Fr 67: 478-482.

Bratina, G; Mortillaro, F. (1966) **[Experimental study of the pulmonary changes induced with urea]. STUDIO SPERIMENTALE SULLE ALTERAZIONI POLMONARI INDOTTE DALL'UREA**. Biol Lat 19: 387-398.

Bulpitt, CJ; Breckenridge, A. (1976) **Plasma urea in hypertensive patients**. Br Heart J 38: 689-694. The annual increase in plasma urea was measured in 253 hypertensive patients. On average there was a significant increase in plasma urea with time which did not depend on the sex of the patient or the type of hypertension. It did, however, depend on the initial level of plasma urea. A table giving the upper limits for expected annual increment may prove useful in clinical assessment. The relation between plasma urea and presenting blood pressure and age was examined in 1217 patients seen at the Hammersmith Hospital hypertension clinic from 1952 to 1967. The plasma urea was significantly related to both age and diastolic and systolic blood pressure. It was higher in men than in women up to 60 years of age, but not above that age, and it increased with presenting mean blood pressure in both sexes, but the increase was greater in men. There was a quadratic relation between age and plasma urea in both men and women. In both sexes the plasma urea increased between the ages of 60 and 80.

Bundy, R; Persaud, C; Jackson, AA. (1993) **Urea kinetics in free-living female vegetarians measured with a single-dose method**. Proc Nutr Soc 52: 12-15.

Bundy R; Persaud C; Jackson AA. (1993) **Measurement of urea kinetics with a single dose of [15N15N]-urea in free-living female vegetarians on their habitual diet**. Int J Food Sci Nutr 44: 253-259. Urea kinetics were estimated in 6 healthy vegetarian women with no restrictions on lifestyle.

Following a single oral dose of [15N15N]-urea, all urine was collected for 48 h and the amounts of [15N15N]-urea and [15N14N]-urea estimated and used to determine the rates of urea production and urea salvage by the colonic microflora. Dietary intake was recorded over the same period and energy and nitrogen intake were derived from food composition tables. There was wide inter-individual variability. Body mass index (BMI) ranged from 18.8 to 25.6 kg/m[sup2], energy intake between 7.1 and 10.7 MJ/day and protein intake between 46.4 and 73.6 g/day. When expressed in relation to body mass, N intake ranged between 106 and 209, urea production between 114 and 248 and urinary urea excretion between 78 and 174 mg kg-1 day-1. The proportion of urea produced which was excreted in urine varied with the protein intake from 50 to 70%, with 30 to 50% of the urea N produced being salvaged in the colon. When the pattern of salvage/production relative to the intake of protein was compared with that seen for 100 reference estimations made in individuals on a mixed omnivorous diet, there was little difference. It is concluded that this method for estimating urea kinetics was suitable for use in free-living individuals. Differences in urea kinetics between vegetarians and omnivores were more likely to be a consequence of differences in the amount rather than the quality of protein ingested.

Cade, JF; Pain, MC. (1972) **Lung function in provoked asthma: responses to inhaled urea, methacholine and isoprenaline**. Clin Sci 43: 759-769.

Carpenter TM. (1938) **The effect of urea on the human respiratory exchange and alveolar carbon dioxide**. J Nutr 15: 499-512. To determine whether the transfer of urea from the blood into the urine is the cause of the S.D.A. of protein and whether the R.Q. is altered by the alkalosis that occurs in humans after ingestion of urea, expts. were made in which the respiratory exchange and the alveolar CO2 were simultaneously measured in consecutive 15-min. periods before and after doses of 30 and 40 gm. of urea. There was no change in the O2 consumption for 3 1/2 hrs. after urea ingestion. Therefore the stimulating effect on the heat production of the eating of protein is not due to the transference and elimination of urea formed as a waste product in protein metabolism. The alveolar CO2 showed a marked increase for at least 2 hrs. after the urea dose, and the maximum elimination of urea-N occurred in the period of maximum change in alveolar CO2. The R.Q., although remaining unchanged for about 1 hr. after urea ingestion, increased in the next 2 hrs. significantly above the empirical quotient in protein combustion. As the alveolar CO2 also rises after ingestion of protein, due to gastric secretion in the stomach, alterations in the R.Q. from the empirical protein quotient are due to the effect of gastric secretion and to the urea ultimately resulting from the metabolic transformations of protein in the body. ABSTRACT AUTHORS: Auth. (courtesy Wistar Bibl. Serv.)

Carraro F; Kimbrough TD; Wolfe RR. (1985) **Urea kinetics in humans at two levels of exercise intensity**. J Appl Physiol 75: 1180-1185. A primed constant infusion of [15N2]urea was used to quantify the response of urea production to exercise at 40 and 70% maximal oxygen consumption on a treadmill. Total urea production, urea production from recycled N, urea production from nonrecycled N, and urea N recycled back into body protein were calculated. Most components of urea kinetics were unaffected by exercise at either intensity. The rate of urea reincorporated into protein was significantly increased during exercise and recovery at both levels of exercise. We conclude that exercise does not stimulate urea production but that there may be an accelerated reincorporation of urea N back into body protein.

Carreon, T; Butler, MA; Ruder, AM; et al. (2005) **Gliomas and farm pesticide exposure in women: the Upper Midwest Health Study**. Environ Health Perspect 113: 546-551. An excess incidence of brain cancer in male farmers has been noted in several studies, but few studies have focused on women. The National Institute for Occupational Safety and Health Upper Midwest Health Study evaluated effects of rural exposures for 341 female glioma cases and 528 controls, all adult (18-80 years of age) nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin. On average, controls lived

longer on farms than did cases. After adjusting for age, age group, education, and farm residence, no association with glioma was observed for exposure to arsenicals, benzoic acids, carbamates, chloroacetanilides, dinitroanilines, inorganics, organochlorines, organophosphates, phenoxys, triazines, or urea-based or estrogenic pesticides. An increased risk of glioma was observed for carbamate herbicides but was not statistically significant (odds ratio = 3.0; 95% confidence interval, 0.9-9.5). No association was observed between glioma and exposure to 12 widely used specific pesticides, after adjustment for age, age group, education, and any other pesticide exposure. These results were not affected after exclusion of proxy respondents (43% of cases, 2% of controls). Women were less likely than men to have applied pesticides, but more likely to have laundered pesticide-contaminated clothes. Storing pesticides in the house was associated with a statistically non-significant increased risk. Results show that exposure to pesticides was not associated with an increased risk of intracranial gliomas in women. Other farm-related factors could be etiologic factors and will be discussed in future reports.

Chantler C. (1994) **What is the significance of the ratio of the plasma urea to the plasma creatinine?** Pediatr Nephrol 8: 536.

Clark PI; Slevin ML; Webb JAW; et al. (1988) **Oral urea in the treatment of secondary tumors in the liver**. Br J Cancer 57: 317-318. Twenty patients with secondary liver tumuors, predominantly from colorectal carcinoma, were treated with oral urea at a daily dose of 8 g m-2. Treatment was well tolerated without side-effects. No objective responses were seen. It is concluded that oral urea is ineffective in the treatment of live metastases from colorectal cancer.

Cramers M; Thormann J. (1981) **Skin reactions to a urea-containing cream**. Contact Dermatitis 7: 189-191. Patch tests with a cream (Calmuril) containing 10% urea were performed on 79 patients with eczematous skin disease; seven (9.9%) had positive tests. Patch tests with the ingredients of the cream were negative. It is suggested that the positive patch test reactions are toxic, due to hypertonicity and acidity of the cream.

Craven PA; Studer RK; Derubertis FR. (1981) **Renal inner medullary prostaglandin synthesis. A calcium-calmodulin- dependent process suppressed by urea**. J Clin Invest 68: 722-732. Previous studies have demonstrated that hyperosmolar NaCl and mannitol stimulate immunoreactive prostaglandin E (iPGE) production by slices of inner medulla (IM), whereas urea inhibits this process. In the present study, the roles of Ca2+ and calmodulin in the control of PGE synthesis in IM and the basis for the differential actions of solutes were examined. A23187 increased [14C]arachidonate (AA) release and iPGE accumulation in the presence but not in the absence of media Ca2+ whereas stimulation by hypertonic NaCl or mannitol was well expressed with Ca2+ or in Ca2+-free buffer containing 2 mM EGTA. Hypertonic urea and trifluoperazine (TFP), an inhibitor of actions of the Ca2+-CaM complex, suppressed increases in [14C]AA release and iPGE induced by A23187, NaCl, or mannitol. By contrast, increases in iPGE in response to exogenous AA were not altered by urea or TFP. Ca2+ (25-100 microM) increased acyl hydrolase (AH) activity in EGTA washed (4 degrees C) 100,000 g particulate fractions of IM threefold, thereby restoring AH activity to the higher basal values of particulate fractions not washed with EGTA. This action of Ca2+ was blocked by hypertonic urea of TFP, whereas AH activity was not influenced by NaCl or mannitol in the presence or absence of Ca2+. In contrast to their effects on AH activity, hypertonic urea and TFP did not alter conversion of AA to PGE2, PGF2 alpha, or PGD2 by IM microsomal fractions. Ca2+-induced increases in particulate AH were blunted after partial depletion of endogenous CaM-like activity. Ca2+ action was restored by addition of purified exogenous CaM, but not by addition of other small acidic proteins, including troponin C. The findings support a role for CaM in the regulation of PGE synthesis in the IM at the level of Ca2+-responsive AH activity. They further imply that urea suppresses PGE synthesis in IM through inhibition of AH and a reduction in the

availability of endogenous AA for conversion to PGE.

Creaven PJ; Madajewicz S; Pendyala L; et al. (1987) **Phase I clinical trial of 1-(2-[2-(4-pyridyl)-2-imidazoline-1-yl]-ethyl)-3-(4-carboxy-phenyl) urea (CGP 15720A)**. Cancer Chemother Pharmacol 20: 145-150. A phase I clinical trial of the intravenous administration of a novel pyridyl imidazoline ethyl carboxy phenyl urea was carried out in 42 patients with advanced solid tumors. Five schedules were evaluated: I, daily X 5; II, daily X 10; III, daily X 15; IV, continuous infusion for 5 days; V, continuous infusion for 7 days. Toxicity was not seen in schedule I (maximum dose 3 g/m2/day) and was minimal in schedule IV (6 g/m2/day). In schedule II it was seen at 2 and 3 g/m2/day, in schedule III at 2 g/m2/day and in schedule V at 6 g/m2/day. Dose-limiting toxicity consisted of a syndrome of lethargy and fatigue. There were no definitely drug-related changes in hematologic or serum chemistry parameters. No responses were seen, but relief of pain in three patients with prostate cancer was noted. Pharmacokinetics indicate a short half-life, limited volume of distribution, and rapid renal clearance. The recommended dose for phase II studies is 3 g/m2/day X 10 or 2 g/m2/day X 15 days.

Dallocchio M; Clementy J; Choussat A; et al. (1974) **[Value of intravenous pyelography combined with the urea washing test in the detection of renalovascular hypertensions (350 cases)]**. Arch Mal Coeur Vaiss 67: 469-480.

Danielsen M; Jackson AA. (1979) **Limits of adaptation to a diet low in protein in normal man: urea kinetics**. Clin Sci 83: 103-108. 1. Urea kinetics were measured using prime/intermittent oral doses of [15N15N]urea in six healthy men taking diets adequate in energy and containing either 74 or 30 g of protein/day. 2. On 74 g of protein/day, urea production (199 mg of N day-1 kg-1) was 121% of intake, with 60% of the urea produced being excreted in the urine and 40% being salvaged in the colon; 69% of the salvaged nitrogen was retained in the metabolic nitrogen pool. 3. Nitrogen balance was not maintained on 30 g of protein/day. There was a significant decrease in the urea production rate (123 mg of N day-1 kg-1) and 54% of production was excreted in urine, with 46% being salvaged. 4. The pattern of urea production and salvaging on 30 g of protein/day was different to that seen in an earlier study on 35 g of protein/day, with a significant decrease in both production (71%) and salvaging (50%). 5. These data reinforce the conclusions drawn from an earlier study, that the salvaging of urea nitrogen by the colon is an integral part of the process of adaptation to low protein diets. The salvage system appears to fail on an intake of 30 g of protein/day and nitrogen is no longer conserved in sufficient amounts for balance to be maintained. 6. The changes seen in urea kinetics reinforce the conclusion based upon nitrogen balance that the minimum physiological requirement for protein in normal adult man lies between 30 and 35 g of protein/day.

Decaux G; Genette F. (1981) **Urea for long-term treatment of syndrome of inappropriate secretion of antidiuretic hormone**. Br Med J (Clin Res Ed) 283: 1081-1083. The efficacy of oral urea in producing a sufficiently high osmotic diuresis was tested in seven patients with the syndrome of inappropriate secretion of antidiuretic hormone. In all patients urea corrected the hyponatraemia despite a normal fluid intake. Five patients were controlled (serum sodium concentration greater than 128 mmol(mEq)/1) with a dose of 30 g urea daily, and two with 60 g daily. The patients who needed 30 g drank 1-2 1 of fluid daily, while those who needed 60 g drank up to 3.1 per day. No major side effects were noted, even after treatment periods of up to 270 days. These findings suggest that urea is a safe and efficacious treatment of the syndrome of inappropriate secretion of antidiuretic hormone.

DeStefano GA; Kahl JB; Hasrajani M; et al. (1969) **Impending gangrene of hand from concentrated urea-sugar solution. Case report**. Plast Reconstr Surg 44: 193-196.

Dolle W; Lahn M. (1972) **[Antabus syndrome under sulfonyl urea treatment]**. Internist (Berl) 13: 425-426.

Dosoo DK; Owusu-Agyei S; Asante KP; et al. (2006) **Pediatric reference ranges for urea and creatinine on the Selectra E Clinical Chemistry Analyzer**. Clin Chem 52: 23-27.

Dossetor JB. (1966) **Creatininemia versus uremia. The relative significance of blood urea nitrogen and serum creatinine concentrations in azotemia**. Ann Intern Med 65: 1287-1299. The physiology of urea and creatinine production by the body and excretion by the kidney is reviewed and contrasted. The normal ratio of B;U.N. [Blood Urea Nitrogen] to serum creatinine is 10:1 and is usually maintained in chronic progressive renal failure. Clinical situations are reviewed in which the ratio is either considerably greater or less than normal. Data from 9 patients confirm that the B.U.N.: creatinine ratio is increased when prerenal factors cause hemodynamic depression in subjects with otherwise moderate renal impairment, when protein intake or breakdown is excessive, and in certain obstructive uropathies. Data also show that the ratio is decreased in situations of low protein intake, tissue anabolism, liver disease or long-term hemodlalysis. The serum creatinine is the more valid index of the renal function. The ratio of B.U.N. to serum creatinine is of considerable diagnostic value when correctly assessed.

El Allaf D; Marchal C; El Allaf M; et al. (1984) **[Development of urea, creatinine and creatinine clearance as a function of age and sex]. EVOLUTION, EN FONCTION DE L'AGE ET DU SEXE, DE L'UREE, DE LA CREATININE ET DE LA CLAIRANCE DE LA CREATININE**. Arch Int Physiol Biochim 92: 249-254. Serum urea and creatinine concentrations were determined in 150 healthy subjects. The formula of Cockcroft and Gault was used in order to calculate creatinine clearance. Such estimation of creatinine clearance is widely used as a parameter for individualization of dosage of drugs secreted primary via the kidneys. The effects of age and sex were then assessed on serum urea, serum creatinine and on creatinine clearance.

El Far, M; El Naggar, M; Elkhawaga, OA; et al. (2006) **Carcinoembryonic antigen, alpha-fetoprotein, and prostate-specific antigen in the sera of industrial workers exposed to phenol, formaldehyde, urea, and mixed vapors**. Inhal Toxicol 18: 1041-1046. Exposure to certain industrial agents has been thought to have carcinogenic potential, both for employees who work closely with agents and for the general population that comes into contact with them. The objective of the present study is to evaluate the changes at the cellular level or at the level of cellular metabolism products present in the biological fluid, and to detect early stages of the carcinogenic process resulting from the exposure of industrial environmental hazards. Carcinoembryonic antigen (CEA), alpha-fetoproteins (AFP), and prostate-specific antigen (PSA) were measured in sera of workers (n = 51), who were divided into 4 groups: group I, workers exposed to phenol; group II, workers exposed to formaldehyde; group III, workers exposed to urea; and group IV, workers exposed to mixed vapor, plus a reference control healthy group (n = 15). The results showed that 75% of the workers exposed to phenol, 75% of the workers exposed to urea, 83.3% of workers exposed to formalin, and 92.3% of the workers exposed to mixed vapors had raised values of serum CEA (S-CEA) above normal value of the control group. Also, 23% of workers exposed to mixed vapors, 44% of workers exposed to formalin, 50% of workers exposed to phenol, and 62.5% of workers exposed to urea had raised values of serum AFP (S-AFP) above normal value of control group. Finally, 16.6% of workers exposed to phenol, 23% of workers exposed to mixed vapors, and 33.3% of workers exposed to formalin had raised values of serum PSA (S-PSA) above the normal value of control group; there were no raised values of S-PSA in workers exposed to urea. No significant difference was found in the activities of AST and ALT in group I, but a highly significant increase was found in the AST activities for groups II and IV and the ALT activities for groups III and IV. A significant difference was found in the activity of ALT in group II and in AST for group III. There was no significant difference in

the levels of albumin in groups I, II, and III, whereas albumin levels were significantly decreased in group IV. No significant change was found in the level of urea and creatinine in all groups except for group III, where serum levels of creatinine were significantly decreased. From our findings, we concluded that S-CEA can be used as an important prognostic screening marker for early prediction for malignancy, and for management of workers with lung cancer who are exposed to the environmental hazards in industrial factories. Furthermore, S-AFP can be used also as a biomarker if it is carried out and correlated with S-CEA.

el-Khoury AE; Fukagawa NK; Sanchez M; et al. (1994) **Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-13C]leucine and [15N-15N]urea**. Am J Clin Nutr 59: 1000-1011. The validity of tracer-derived estimates of whole-body leucine balance was investigated. Seven healthy young adult subjects received an adequate protein diet for 6 d; at 1800 on the last day, L-[1-13C]leucine and [15N-15N]urea were given as primed, continuous intravenous infusions for 24 h. Subjects were in a fasting state for the first 12 h and at 0600 on day 7 they then received hourly 10 equal meals to achieve a fed state. Total leucine intake (diet plus tracer) was 89.4 mg. kg-1.d-1. Mean daily leucine oxidation was equivalent to 89.5 +/- 3.3 mg leucine/kg. The predicted daily oxidation rate, from measurements made during the last hour of the fast and the fifth hour of the fed period, was 91.2 +/- 5.8 mg/kg (P = 0.25 from measured). Measured and predicted whole-body leucine balances were 0.76 +/- 2.99 and -0.98 +/- 5.54 mg/kg, respectively (P = 0.25). Urea production exceeded urea excretion by 20%; daily protein oxidation was the same when estimated from leucine oxidation or nitrogen excretion. Thus, the tracer-balance concept is valid, and reliable predictions of total daily leucine oxidation and whole-body leucine balance can be obtained from short-term measurements of leucine oxidation during fasted and fed states.

Fisher AA. (1976) **Irritant reactions from topical urea preparations used for dry skin. Advantages of a urea-free "Dead Sea salt" cream**. Cutis 18: 761, 763, 767 passim.

Fouillet H; Bos C; Mariotti F; et al. (2004) **Effect of increasing habitual protein intake on dietary nitrogen metabolic fate and urea recycling in humans: a compartmental analysis**. FASEB J 18: 4-5. A new 13-compartment model was developed to simulate the dietary nitrogen (N) distribution and metabolism in the postprandial non-steady state in humans. It was built and validated from experimental data obtained in subjects adapted for 7d to normal (NP: 1 g/kg/d) and for the next 7d to high (HP: 2 g/kg/d) protein diets. After each period, the subjects ingested a fixed dose of ¹⁵N-labeled soy protein and energy as a single liquid, mixed meal. Dietary N kinetics were measured in plasma proteins and amino acids, body urea, urinary urea and ammonia during 8h after the meal ingestion. Our model showed that adaptation to HP diet delayed gastric emptying of the same meal N (half-time increased from 69 to 79 min). In the splanchnic bed, meal N transfer to urea increased by 50% after adaptation to HP diet concurrently with a similar incorporation of dietary N into splanchnic protein compared to NP diet. This led to a subsequent reduced uptake and anabolic utilization (-25%) of meal N by the peripheral area after HP vs. NP diet. Furthermore, 6h after the meal, 19 % and 11 % of the urea N produced from meal N was reincorporated into splanchnic amino acids after NP and HP, respectively. This result evidences a relative reduction of dietary N recycling from urea in response to increasing habitual protein intakes. Such a model constitutes a useful, explanatory tool to describe the impact of diets on the homeodynamic response to ingestion.

Fure S; Lingstrom P; Birkhed D. (1998) **Effect of three months' frequent use of sugar-free chewing gum with and without urea on calculus formation**. J Dent Res 77: 1630-1637. Studies on the relationship between gum-chewing and calculus formation have produced contradictory results, and it is not clear whether frequent use of chewing gum promotes or inhibits calculus formation. Also, little is

known about whether the addition of a small amount of urea to the chewing gum influences calculus formation. The aim of this investigation was to study the effect of sugar-free chewing gum--with and without urea--on calculus formation and some associated clinical variables. Three three-month periods were studied in a double-blind, crossover design, during which the subjects: (1) chewed 5 pieces/day of a sugar-free, urea-containing chewing gum (20 mg urea/piece); (2) chewed 5 pieces/day of a sugar-free, non-urea-containing gum; or (3) performed no gum-chewing. Twenty-nine persons, all calculus-formers, participated. They were scored for calculus at mesio-lingual, lingual, and disto-lingual sites on the 6 anterior mandibular teeth according to the Volpe-Manhold index. Plaque and gingival bleeding index, stimulated salivary secretion rate and buffer capacity, resting plaque pH, mutans streptococci in saliva and plaque, and lactobacilli in saliva were also determined. No differences in calculus formation were found among the 3 periods. The resting plaque pH was higher after the period with urea-containing gum than after the period with non-urea-containing gum and the no-gum period ($p < 0.05$). A slight increase in stimulated salivary secretion rate was found after the 2 gum periods ($p < 0.05$). The plaque and gingival bleeding indices decreased, while resting plaque pH and salivary buffer capacity increased throughout the entire study ($p < 0.05$). No significant differences in prevalence of the acidogenic micro-organisms were found among the test periods. The main conclusion from this study is that three months' frequent use of sugar-free chewing gum--with or without urea--neither promotes nor inhibits calculus formation.

Gandhi GM; Anasuya SR; Kawathekar P; et al. (1977) **Urea in the management of advanced malignancies (preliminary report)**. J Surg Oncol 9: 139-146. Twenty cases of advanced (Stage III) cancer of the cervix were treated with intratumour injection of 40% urea solution and local application of 50% urea ointment. Sixty percent of these patients had beneficial effect and in 25% of the patients there was minimal response. Patients with multiple secondaries in liver had very good symptomatic relief with oral urea. Urea therapy is a simple, cheap, and safe method of treating advanced stages of cancer, where very little can be done by the accepted lines of treatment such as surgery or radiotherapy. This line of treatment can be practiced even in a consulting room or primary health center.

Geiseler D; Kay JD; Oberholzer VG; et al. (1979) **Determination of the rate of urea synthesis from serial measurements of plasma urea concentration after an alanine load: theoretical and methodological aspects**. Clin Sci 68: 201-208. A method is described by which the rate of synthesis of urea can be calculated from the change of plasma concentration of urea after an alanine load. The results can be expressed in terms of f, the maximum increase in the rate of urea synthesis, and t, the time at which urea synthesis reaches its maximum. These parameters are calculated by an algebraic curve-fitting technique which is suitable for a desk computer. The method removes the need for isotopic analysis and urine collections. The effect of various errors and experimental conditions on the calculated synthesis parameters is investigated.

Gindler EM. (1970) **Nomograms for calculation of urea clearance**. Clin Chemi 16: 347-349.

Giordano C. (1963) **Use of exogenous and endogenous urea for protein synthesis in normal and uremic subjects**. J Lab Clin Med 62: 231-246.

Gobbato F. (1956) **[Relation of oxygen consumption to azotemia and clearance of urea; illustration of clinical application of this relationship]. RELAZIONE TRA CONSUMO PROTEICO, AZOTEMIA E CLEARANCE UREICA; NOMOGRAMMA CHE ILLUSTRA LE APPLICAZIONI CLINICHE DI TALE RELAZIONE**. Minerva Nefrol 3: 60-61.

Goldstein MH; Lenz PR; Levitt MF. (1969) **Effect of urine flow rate on urea reabsorption in man: urea as a "tubular marker"**. J Appl Physiol 26: 594-599.

Gross A; Gaultier J; Mangin JP. (1965) **[Variations in Humans of Urea Clearance as a Function of Urinary Output. The Rheumaturic Coefficient]. DES VARIATIONS, CHEZ L'HOMME, DE LA CLEARANCE UR'EIQUE EN FONCTION DU D'EBIT URINAIRE. LE COEFFICIENT RHEUMATURIQUE**. J Physiol (Paris) 57: 248.

Hamadeh MJ; Hoffer LJ. (1998) **Tracer methods underestimate short-term variations in urea production in humans**. Am J Physiol 274: E547-53. Urea production rate (Ra) is commonly measured using a primed continuous tracer urea infusion, but the accuracy of this method has not been clearly established in humans. We used intravenous infusions of unlabeled urea to assess the accuracy of this technique in normal, postabsorptive men under the following four different conditions: 1) tracer [13C]urea was infused under basal conditions for 12 h (control); 2) tracer [13C]urea was infused for 12 h, and unlabeled urea was infused from hours 4 to 12 at a rate twofold greater than the endogenous Ra ("step" infusion); 3) tracer [13C]urea was infused for 12 h, and unlabeled urea was infused from hours 4 to 8 ("pulse" infusion); and 4) tracer [13C]urea was infused for 9 h, and unlabeled alanine was infused at a rate of 120 mg.kg-1.h-1 (1.35 mmol.kg-1.h-1) from hours 4 to 9. Urea Ra was calculated using the isotopic steady-state equation (tracer infusion rate /tracer-to-tracee ratio), Steele's non-steady-state equation, and urinary urea excretion corrected for changes in total body urea. For each subject, endogenous urea Ra was measured at hour 4 of the basal condition, and the sum of this rate plus exogenous urea input was considered as "true urea input". Under control conditions, urea Ra at hour 4 was similar to that measured at hour 12. After 8-h step and 4-h pulse unlabeled urea infusions, Steele's non-steady-state equation underestimated true urea input by 22% (step) and 33% (pulse), whereas the nonisotopic method underestimated true urea input by 28% (step) and 10% (pulse). Similar conclusions were derived from the alanine infusion. These results indicate that, although Steele's non-steady-state equation and the nontracer method more accurately predict total urea Ra than the steady-state equation, they nevertheless seriously underestimate total urea Ra for as long as 8 h after a change in true urea Ra.

Hamberg O; Vilstrup H. (1994) **A rapid method for determination of hepatic amino nitrogen to urea nitrogen conversion ('the Functional Hepatic Nitrogen Clearance ')**. Scand J Clin Lab Invest 54: 377-383. The Functional Hepatic Nitrogen Clearance (FHNC) is a measure of the functional liver mass as to conversion of amino-N to urea-N. FHNC is the slope of the linear regression of multiple samples (10-20) of urea-N synthesis rates (UNSR) on blood alpha-amino-N concentrations (alpha-AN) during infusion of amino acids. UNSR is measured as urinary urea-N excretion rate corrected for accumulation in total body water (TBW) and loss in gut. A simplified method which estimates FHNC from only two samples of UNSR and alpha-AN was developed. Urine was collected in two hourly intervals: before infusion of alanine, and from 2 to 3 h after start of alanine infusion. Blood- urea-N and alpha-amino-N was measured at the beginning and at the end of each urine sampling interval. TBW was estimated from a nomogram, and gut loss of urea was assigned a fixed value (14%). The two-sample FHNC was calculated as delta UNSR (mmol h-1)/delta mean alpha-AN (mmol l-1). Linear regression analysis of the two-sample estimates of FHNC on the 'true' multiple-sample values of FHNC in an independent population of control and cirrhotic subjects showed the two-sample estimates to be closely related with values of the multiple-sample method, the regression equation being: two-sample FHNC = -0.24 + 0.99 x multiple-sample FHNC, r2 = 0.98. A close relationship was also obtained when cirrhotic patients were considered alone: two-sample FHNC = 0.01 + 0.94 x multiple-sample FHNC, r2 = 0.98.(ABSTRACT TRUNCATED AT 250 WORDS)

Hamberg O; Vilstrup H. (1994) **Regulation of urea synthesis by glucose and glucagon in normal man**. Clin Nutr 13: 183-191. The separate effects of glucose and glucagon on alanine stimulated hepatic amino-N to urea-N conversion, quantified by the Functional Hepatic Nitrogen Clearance (FHNC) (i.e. the linear slope of the relation between urea synthesis rate and blood alpha-amino-N concentration), were

studied in 7 healthy subjects. FHNC was measured four times in each: during constant infusion of alanine alone; alanine superimposed on constant glucose infusion; alanine superimposed on glucose and low stepwise glucagon infusions; and alanine superimposed on glucose and high constant glucagon infusions. Glucose halved the glucagon response to alanine. This reduction was abolished by the low stepwise glucagon infusion, aimed at re-establishing portal glucagon levels. The high glucagon infusion resulted in 3-fold elevated glucagon levels. During alanine infusion alone FHNC was (mean +- SEM) 32.5 +- 1.9 l/h. Glucose reduced FHNC by 43% to 18.4 +- 0.9 l/h (p lt 0.01). The low stepwise glucagon infusion only partially normalized FHNC as reduced by glucose (to 24.6 +- 1.5 1/h, (p lt 0.01 vs alanine alone)). The high glucagon infusion increased FHNC by 35% despite hyperglycaemia (to 44.1 +- 1.5 l/h, (p lt 0.01 vs alanine alone)). The results show that both glucose and glucagon are independent but opposite regulators of hepatic amino-N conversion. The physiological glucose effect is accomplished by a combination of both the effect of glucose itself and the inhibition by glucose of the glucagon response to alanine. Hyperglucagonaemia increases FHNC and overrules the inhibition by glucose. This may explain the defect nitrogen sparing by glucose and to some extent the catabolism in hyperglucagonaemic stress conditions, despite prevailing hyperglycaemia.

Heine W; Tiess M; Stolpe HJ; et al. (1984) **Urea utilization by the intestinal flora, of infants fed mother's milk and a formula diet, as measured with the 15N-tracer technique**. J Pediatr Gastroenterol Nutr 3: 709-712. 15N-Incorporation by intestinal bacteria was measured under different feeding conditions in 16 infants after a single oral loading of 165 mg [15N2]urea X kg-1 body weight as a tracer. In five subjects on a mother's milk diet, the 15N-excess in the isolated intestinal bacteria was 1.08 (0.17-1.85) atom-%. The mean 15N-excess in the intestinal flora of five formula-fed subjects did not differ significantly from these values [0.63 (0.17-1.05) atom-%]. A trend to a higher incorporation of 15N from labeled urea by the intestinal flora was seen in four infants, who were adapted to an increased nutritional urea supply on a special formula, containing 14 g of milk protein, 80 g lactose, 36 g fat, and 0.35 g urea X L-1. The same observation was made in two infants with chronic renal failure. The incorporation of urea nitrogen by the putrefactive intestinal flora of infants on a formula diet as well as by the bifidobacterial flora of those on mother's milk feeding indicates the utilization of ureas as a source of bacterial protein and nucleic acid synthesis. The adaptive usage of urea for the bacterial metabolism can be considered as a sign of supportive detoxification by the intestinal flora.

Hernandez P; Castro R; Portuondo H; et al. (1972) **[Use of intravenous urea in the treatment of the patinful crises of sickle cell anemia]**. Rev Invest Clin 24: 83-89.

Hibbert JM; Jackson AA. (1991) **Variation in measures of urea kinetics over four years in a single adult**. Eur J Clin Nutr 45: 347-351. Urea kinetics was measured in a single woman on five occasions over four years by the prime/intermittent oral-dose method with [15N15N]-urea. On a nitrogen intake of 231 +/- 24 mg/kg/day, urea production was 198 +/- 22 mg/kg/day, with the urinary excretion of urea being 143 +/- 25 mg/kg/day. Urea hydrolysis and salvaging in the bowel was 55 +/- 6 mgN/kg/day. The coefficients of variation for production, 11 per cent, excretion, 18 per cent, and hydrolysis, 11 per cent, were similar to that for intake, 10 per cent, and substantially less than reported inter-individual variations from other studies. It is concluded that the method employed for measuring urea kinetics gives reproducible results and that the intra-individual variation in urea kinetics is much less than the inter-individual variation.

Higgins C. (1994) **Measuring renal function with urea and creatinine tests**. Nurs Times 90: 34-36. The second paper in our series on laboratory examination of specimens discusses the measurement of urea and creatinine in the blood as tests of renal function. It outlines the synthesis of urea and creatinine and their excretion following glomerular filtration.

Himmelfarb J. (1999) **Urea: surrogate or toxin?** Kidney Int 56: 754-755.

Ivarsen P; Greisen J; Vilstrup H. (1979) **Acute effects of moderate dehydration on the hepatic conversion of amino nitrogen into urea nitrogen in healthy men**. Clin Sci 101: 339-344. Intracellular hydration may play a role in the regulation of protein and nitrogen metabolism. The hepatic removal of nitrogen by urea synthesis has a key regulatory role in nitrogen balance. The purpose of the present study was to establish the acute effects of dehydration on the hepatic kinetics of urea synthesis, quantified by functional hepatic nitrogen clearance (FHNC), in healthy volunteers. Seven healthy men were studied twice in random order. On both study days, a primed continuous infusion of alanine was given. On the day of dehydration an intravenous bolus injection of a loop diuretic (furosemide, 1 mg/kg) was superimposed. FHNC was calculated as the ratio between measured synthesis rates of urea nitrogen and blood alanine concentrations. Furosemide induced a weight loss of 1 kg. During dehydration, FHNC decreased by approx. 25% (41+/-11 to 54+/-10 litres/h; P<0.02). On both occasions individual FHNC and glucagon values were positively correlated (r(2)>0.6). In addition, dehydration more than halved the linear slope of the relationship (P<0.05). The FHNC values were correlated with the urinary excretion of both potassium and sodium (r(2)=0.68, P<0.01 and r(2)=0.62, P<0.02 respectively). Changes in the reactivity of urea synthesis to glucagon (i.e. the ratio between FHNC and glucagon concentration) was negatively correlated with an indirectly estimated change in intracellular water (r(2)=0.79, P<0.05). We conclude that acute moderate dehydration down-regulates both total urea synthesis and its sensitivity to glucagon. The latter was related to estimated intracellular water loss. Dehydration may thus have nitrogen-saving consequences with regard to the hepatic contribution to whole-body nitrogen homoeostasis. The mechanism of this effect and the relationship with sodium and potassium fluxes is not known.

Jackson AA; Danielsen MS; Boyes S. (1993) **A noninvasive method for measuring urea kinetics with a single dose of [15N15N]urea in free-living humans**. J Nutr 123: 2129-2136. A noninvasive method is described in which the endogenous rate of urea production can be determined in normal, free-living adults. A single dose of [15N15N]urea was given orally, and the amount of label excreted as [15N15N]urea and [15N14N]urea in urine over the subsequent 48 h was measured. From the rates of excretion of labeled and unlabeled urea the rate of urea production was derived. Using this single- dose protocol the rate of urea production was 207 +/- 56 (mean +/- SD) mg N/(kg.d) in six normal adult men consuming 74 g protein/d. These results were not different when compared with rates of urea production obtained with a prime/intermittent protocol in an earlier study in the same individuals [199 +/- 20 mg N/(kg.d)]. We conclude that urea kinetics can be measured noninvasively with a single dose of [15N15N]urea and that this method may be suitable for use in free-living individuals to determine urea production rates for habitual dietary intakes.

Jackson AA; Picou D; Landman J. (1984) **The non-invasive measurement of urea kinetics in normal man by a constant infusion of 15N15N-urea**. Hum Nutr Clin Nutr 38: 339-354. A two-pool model is described for the non-invasive measurement of urea kinetics in man. The isotope, 15N15N-urea , was given until an isotopic steady state was reached in urine and the time taken to achieve this is defined. During an isotopic steady state, a comparison was made of the effect of giving the isotope orally, intravenously and intragastrically; no differences were found between the different routes. Measurements of enrichment were made on excretion products in urine. In six normal adults with a protein intake of 200 mg N/kg/d, the urea production rate was 139 +/- 15 mg N/kg/d, 70 per cent of which was excreted in urine. Of the 34 mg N/kg/d produced by hydrolysis of urea in the gastrointestinal tract, 41 per cent was resynthesized to urea, and about 48 per cent was available for other synthetic processes.

Jackson AA; Scrimshaw NS; Schurch B. (1991) **Critique of protein-energy interactions in vivo: urea**

kinetics. Protein Energy Interactions: Proceedings of an I/D/E/C/G Workshop held in Waterville Valley, NH, USA, October 21: 63-79. The interaction between energy and protein within the body varies with the functional metabolic demand. The metabolic demand for energy is measured as the flow of carbon through the body, and the main determinant of variability within and between individuals is the level of physical activity. The metabolic demand for protein is measured as the flow of nitrogen through the body and the main determinant of variability is the rate of growth. The demands for N and C often, but not always, move together in the same direction. At marginal levels of energy intake, positive N balance may be defended in the face of a negative energy balance. N balance represents only a fraction of the intensity of the movement of N within the body, as there are 2 major internal cycles for N. The first, characterized as protein turnover, represents the movement of N as amino acids into and from proteins. The intensity and pattern of this movement vary with the pattern of the metabolic demand. The second, less clearly recognized, represents the movement of N from amino acids into urea, and the return of the urea N to amino acid synthesis. The return of the urea N requires the salvaging of N through the metabolic activity of the colonic microflora. Within the range of adequate protein intakes, the production of urea is not related to protein intake. The achievement of N balance seems to depend on the salvage of urea N, implying that the activity of the colonic microflora is an integral part of the mechanism through which N balance is normally maintained. N, amino acids and protein are not terms which can be used casually or interchangeably, and the movement of N through the body can be measured directly only with the use of N labels and not imputed indirectly from the use of C-labelled amino acids.

Jahoor F; Wolfe RR. (1987) **Reassessment of primed constant-infusion tracer method to measure urea kinetics**. Am J Physiol 252: E557-64. The validity of the primed constant-infusion tracer technique to make short-term measurements of urea production rates (Ra) in humans in a physiological steady state and during disruption of steady state was evaluated. Four subjects received a primed constant infusion (P/I = 560 min) of [13C]urea for 8 h. A plateau in urea enrichment was reached after 2 h and maintained throughout. When [13C]- and [18O]urea were simultaneously infused into four subjects at P/I ratios of 560:1 and 360:1, respectively, both tracers reached plateau enrichment at the same time (2-4 h). The enrichment at plateau was a function of the infusion rate rather than the priming dose, and calculated urea Ra was the same with either prime. In five additional experiments the technique responded acutely to a physiological perturbation (alanine infusion) in a dose -dependent manner. The results confirm that this technique is appropriate for short-term measurements of urea Ra, and the requirement for accuracy in estimating the priming dose is not impractically stringent.

Jahoor F; Wolfe RR. (1987) **Regulation of urea production by glucose infusion in vivo**. Am J Physiol 253: E543-50. We have investigated the acute in vivo regulation of urea production in normal postabsorptive volunteers by administering a primed constant infusion of 15N2-urea to measure urea production during the constant intravenous infusion of equivalent molar quantities of exogenous nitrogen, given as alanine or glutamine, either with or without a simultaneous infusion of glucose at 4 mg .kg-.min-1. These responses were compared with the response to the infusion of glucose alone. Both amino acid infusions elicited significant (P less than 0.05) and identical (26%) increases in urea production over 4 h. When the glucose infusion was added to the amino acid infusions, urea production remained constant, despite the comparable increases in plasma total nonessential amino nitrogen, as were observed with the amino acid infusions alone. Glucose infused alone elicited a significant (P less than 0.05) reduction (18%) in urea production but no corresponding change in plasma total amino nitrogen. We conclude that 1) infused glucose or its hormonal response suppresses urea production by blunting the normal hepatic ureagenic response to a fixed nitrogen load, 2) this suppressive effect is not mediated via a reduction in substrate (nitrogen) supply, and 3) the inhibition of hepatic gluconeogenesis from amino acids represents one component of this suppressive effect, and direct suppression of urea cycle activity probably represents another component.

Johnson WJ; Hagge WW; Wagoner RD; et al. (1972) **Effects of urea loading in patients with far-advanced renal failure**. Mayo Clin Proc 47: 21-29.

Karsai T; Menes A; Molnar J; et al. (1979) **Determination of enzyme activity by chromatography and videodensitometry. II. Urea cycle enzymes in tissue homogenates**. Acta Biochim Biophys 14: 133-142. Methods are described for the determination of the activity of urea cycle enzymes in human and rat tissues by chromatography and videodensitometry(CV-technique). With specific substrates carbamoyl-phosphate synthetase and ornithine carbamoyltransferase activities were determined as the amounts of citrulline formed. Argininosuccinate synthetase, argininosuccinate lyase and arginase activities were measured from the changes in ornithine concentration. For measuring the activity of five enzymes 5 to 10 mg wet weight of tissue was sufficient. The CV-technique could be conveniently applied for the investigation of enzyme content in samples from human biopsy.

Kay JD; Seakins JW; Geiseler D; et al. (1979) **Validation of a method for measuring the short-term rate of urea synthesis after an amino acid load**. Clin Sci 70: 31-38. The response of the plasma concentration of urea to the oral and intravenous administration of alanine was studied in healthy adult humans. The instantaneous rate of urea synthesis was calculated by using a model-dependent procedure. The errors in this procedure were calculated and it was shown that analytical precision and sampling frequency, and the estimates of the distribution volume and elimination fluxes, were adequate to determine the synthesis parameters. A direct test of the compartmental model was made by the intravenous injection of exogenous urea. The one-compartment model with first-order elimination gave a good fit to the experimental results at times greater than 8 min after the injection. Both oral and intravenous loads of alanine had dose-dependent effects on the rate of urea synthesis. There was no evidence of a limit to the maximum possible rate of urea synthesis in these experiments and the values obtained were similar to published results for different stimuli and methods of measurement. The rate of synthesis increased more rapidly after intravenous loads and subjective side-effects were less severe. The intravenous administration of alanine appears to be a suitable stimulus for urea synthesis.

Keast RSJ; Breslin PAS. (2002) **Cross-adaptation and bitterness inhibition of L-tryptophan, L-phenylalanine and urea: further support for shared peripheral physiology**. Chem Senses 27: 123-131. A previous study investigating individuals' bitterness sensitivities found a close association among three compounds: L-tryptophan (L-trp), L-phenylalanine (L-phe) and urea (Delwiche et al., 2001, Percept. Psychophys. 63, 761-776). In the present experiment, psychophysical cross-adaptation and bitterness inhibition experiments were performed on these three compounds to determine whether the bitterness could be differentially affected by either technique. If the two experimental approaches failed to differentiate L-trp, L-phe and urea's bitterness, then we may infer they share peripheral physiological mechanisms involved in bitter taste. All compounds were intensity matched in each of 13 subjects, so the judgments of adaptation or bitterness inhibition would be based on equal initial magnitudes and, therefore, directly comparable. In the first experiment, cross-adaptation of bitterness between the amino acids was high (>80%) and reciprocal. Urea and quinine-HCl (control) did not cross-adapt with the amino acids symmetrically. In a second experiment, the sodium salts, NaCl and Na gluconate, did not differentially inhibit the bitterness of L-trp, L-phe and urea, but the control compound, MgSO(4), was differentially affected. The bitter inhibition experiment supports the hypothesis that L-trp, L-phe and urea share peripheral bitter taste mechanisms, while the adaptation experiment revealed subtle differences between urea and the amino acids indicating that urea and the amino acids activate only partially overlapping bitter taste mechanisms.

Khadzhimetov AA; Sultanov SN. (1989) **Transerythrocytic concentrations of urea and the danger in interruption of pregnancy**. Med Zh Uzb: 15-16. In pregnant women under risk of interruption of

pregnancy and the healthy pregnant women the level of urea was 13.3 and 3.31 mM/L, resp., in erythrocytes and 4.46 and 3.01 mM/L, resp., in blood serum. In pregnancy complicated by toxicosis, especially severe form, the level of urea in erythrocytes was 16.9 mM/L (5.71 mM/L in blood serum). Treatment with spasmolytics and tocopherol acetate significantly decreased the level of urea in erythrocytes (3.79 mM/L). It is suggested that tocopherol acetate maintains the membrane structures and improves the maternal-placental circulation.

Kloppenburg WD; Wolthers BG; Stellaard F; et al. (1979) **Determination of urea kinetics by isotope dilution with [13C]urea and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) analysis**. Clin Sci 93: 73-80. 1. Stable urea isotopes can be used to study urea kinetics in humans. The use of stable urea isotopes for studying urea kinetic parameters in humans on a large scale is hampered by the high costs of the labelled material. We devised a urea dilution for measurement of the distribution volume, production rate and clearance of urea in healthy subjects and renal failure patients using the inexpensive single labelled [13C]urea isotope with subsequent analysis by headspace chromatography-isotope ratio MS (GC-IRMS) of the [13C]urea enrichment. 2. The method involves measurement of the molar percentage excess of [13C]urea in plasma samples taken over a 4 h period after an intravenous bolus injection of [13C]urea. During the sample processing procedure, the plasma samples together with calibration samples containing a known molar percentage excess of [13C]urea are acidified with phosphoric acid to remove endogenous CO2, and are subsequently incubated with urease to convert the urea present in the plasma samples into CO2. The 13C enrichment of the generated CO2 is analysed by means of GC-IRMS. This method allows measurement of the molar percentage excess of [13C]urea to an accuracy of 0.02%. 3. Reproducibility studies showed that the sample processing procedure [within-run coefficient of variation (CV) < 2.8% and between-run CV < 8.8%] and the GC-IRMS analysis (within-day CV < 1.3% and between-day CV < 1.3%) could be repeated with good reproducibility. 4. In clinical urea kinetic studies in a healthy subject and in a renal failure patient without residual renal function, reproducible values of the distribution volume, production rate and clearance of urea were determined using minimal amounts of [13C] urea (25-50 mg). 5. Because only low [13C]urea enrichments are needed in this urea dilution method using GC-IRMS analysis, the costs of urea kinetic studies are reduced considerably, especially in patients with renal failure.

Knoll E; Wisser H; Rebel FC. (1978) **[Dependence of the serum concentrations of creatinine and urea on the time of day, with normal and impaired kidney function]. ABHANGIGKEIT DER KONZENTRATIONEN VON KREATININ UND HARNSTOFF IM SERUM VON DER TAGESZEIT BEI NORMALER UND EINGESCHRANKTER NIERENFUNKTION**. J Clin Chem Clin Biochem 16: 567-570. Daily variations in the serum concentrations of creatinine, urea and cortisol were studied in 7 patients without kidney or muscle disease, and in 8 patients with impaired kidney function (creatinine clearance < 40 ml/min). Neither creatinine nor urea showed a circadian rhythm; the daily variations lay within the limits of the methodological scatter. Serum cortisol showed its known circadian rhythm in high amplitude.

Koch CD; Arnst E; Rommel K. (1980) **[Urea and creatinine levels and clearances: observations in 25 healthy subjects for one year (author's transl)]. HARNSTOFF, KREATININ, HARNSTOFF- UND KREATININ-CLEARANCE: UNTERSUCHUNGEN AN 25 GESUNDEN PROBANDEN UBER EIN JAHR**. J Clin Chem Clin Biochem 18: 423-429. The seasonal, intra- und interindividual variation of the creatinine and urea concentrations in serum and urine and the clearances of these compounds were examined monthly for one year in 25 healthy volunteers. In contrast to the other parameters (serum urea, clearance and excretion of creatinine and urea), the variations in serum-creatinine concentration were small and statistically unsignificant. The variations of the urinary excretion and the clearance of creatinine and urea is due to seasonal variations in the output of the kidney.

Kotova, NI. (1986) **Materials for substantiating the maximum permissible concentration of urea in the air of the working zone**. Gig Tr Prof Zabol 3: 43-44. Urea (I) [57-13-6] is a moderately dangerous chemical; its recommended maximum allowable concentrate is 10 mg/m3. This result was obtained in a clin. study of 67 industrial workers in daily contact with I and also in studies on laboratory animals. The breathing of I-containing air caused disturbance in the protein metabolism, moderate emphysema, and, in chromic exposure, loss of weight

Kraus LM; Gaber L; Handorf CR; et al. (2001) **Carbamoylation of glomerular and tubular proteins in patients with kidney failure: A potential mechanism of ongoing renal damage**. Swiss Med Wkly 131: 139-145. Cyanate formed spontaneously from urea carbamoylates non-protonated amino groups of protein, irreversibly altering function, charge and structure. Carbamoylated proteins in renal tissue were not examined hitherto. Homocitrulline was studied, (?-amino-carbamoyl-lysine), a result of in vivo carbamoylation by urea-derived cyanate, from patients with renal disease or in newly transplanted kidneys by immunohistochem. The enzymic activity of carbamoylated and non-carbamoylated matrix metalloproteinase-2 was evaluated and correlated with renal tissue carbamoylated in vivo. Anti-homocitrulline antibody is specific for homocitrulline and was used to identify carbamoylation of ?-amino-lysine in renal biopsies from patients with elevated BUN, with isolated proteinuria, and as controls, from normal donors at time of transplantation. Enzymic activity of matrix metalloproteinase-2 carbamoylated in vitro was evaluated. Homocitrulline was present in glomerular basement membrane (8/10), mesangium (8/10), tubular epithelium and cytoplasm (7/10) and Bowman's capsule (1/10) in patients with elevated BUN. The discordant patterns of glomerular and tubular localization of homocitrulline vs. immune complexes indicated that the carbamoylated proteins were not a component of immune deposits but were modified proteins in renal tissue. No homocitrulline was found in transplanted kidneys (14/15) or in proteinuric patients (2/2). Enzymic activity of both human and rat matrix metalloproteinase-2 was strongly inhibited in a dose-dependent fashion when incubated with cyanate. In situ carbamoylation in proteins occurred in kidneys of patients with renal dys-function but not in normal newly transplanted kidneys. Decreased enzymic activity of carbamoylated enzymes may alter specific renal regulatory mechanisms. Carbamoylated proteins with altered function and charge may represent a previously underestimated mechanism in renal pathophysiol.

Kuster W; Bohnsack K; Rippke F; et al. (1998) **Efficacy of urea therapy in children with ichthyosis. A multicenter randomized, placebo-controlled, double-blind, semilateral study**. Dermatology 196: 217-222. BACKGROUND: Ichthyoses are genetic disorders of keratinization which are uncomfortable due to their conspicuous scaling, itching and cosmetic problems. Especially in childhood, ichthyoses can lead to social discrimination and psychological problems. Efficient therapies are necessary which are safe and well tolerated. OBJECTIVE: The aim of the study was to investigate the keratolytic and moisturizing properties as well as the tolerance of a new urea lotion when applied to hyperkeratotic and ichthyotic skin in childhood. METHODS: The study was conducted as a multicenter, randomized, placebo-controlled, double-blind, semilateral investigation. Sixty children between 1 and 16 years treated one side of the most affected extremity with Laceran 10% urea lotion for 8 weeks. On the other side the urea-free Laceran lotion base was given. On each side of the body a control area was left untreated. The investigators evaluated the global severity of ichthyotic symptoms with the help of a visual analogue scale. RESULTS: The analysis of the global estimation of severity of ichthyosis showed improvements being stronger in the body areas treated with Laceran 10% urea lotion (from 4.8 to 2.0 points) than in the areas treated with the urea-free Laceran lotion base (from 4.8 to 2.5 points). The response rates were 65% after 4 weeks and 78% after 8 weeks for Laceran 10% urea lotion, 50% after 4 weeks and 72% after 8 weeks for the urea-free Laceran lotion base. CONCLUSION: It can be ascertained that Laceran 10% urea lotion has a strong positive effect on generalized ichthyotic keratinization disorders.

Kwan JT; Carr EC; Barron JL; et al. (1993) **Carbamylated haemoglobin--a retrospective index of time-averaged urea concentration**. Nephrol Dial Transplant 8: 565-567.

Lee, JA; Lee, HA; Sadler, PJ. (1991) **Uraemia: is urea more important than we think?** Lancet 338: 1438-1440. Urea is accumulated as an osmolyte by some groups of animals even though it impairs protein function. These organisms can withstand high internal urea concentrations because they also accumulate other low-molecular-weight osmolytes, the methylamines, which can offset the effects of urea on proteins. Methylamines have also been found in the medulla of the mammalian kidney (where urea concentrations are high) and in the plasma of human subjects with chronic renal failure. These findings suggest that previous investigations of the potential contribution of urea to the syndrome of uraemia may have been confounded because of the presence of variable concentrations of protective substances. That naturally occurring methylamines or related substances may prove to have a useful therapeutic role in uraemia is also possible.

Lemiere, C; Malo, JL; Garbe-Galanti, L. (1996) **[Bronchial irritation syndrome following inhalation or urea. Histologic and immunohistochemical evaluation]. SYNDROME D'IRRITATION BRONCHIQUE CONSECUTIF A L'INHALATION D'UREE. EVALUATION HISTOLOGIQUE ET IMMUNO-HISTOCHIMIQUE**. Rev Mal Respir 13: 595-597. We herein report the case of a subject exposed to urea fumes. After exposure, the subject immediately experienced throat and chest burning. A few hours later, she had cough, dyspnea and wheezing during exercise. The functional pulmonary testing performed two months later showed non-specific airway responsiveness. Bronchoscopy with broncho-alveolar lavage and bronchial biopsies was performed. Biopsies showed injury of the epithelial layer that was atrophic and devoid of ciliated cells. There was fibrosis of connective tissue as well as an inflammatory infiltrate. Immunohistochemistry stains showed that most of the inflammatory cells were T-lymphocytes. There were no degranulated eosinophils. The subject was given inhaled steroids. Four months later, bronchial responsiveness was normal.

Loden M; Andersson AC; Lindberg M. (1999) **The effect of two urea-containing creams on dry, eczematous skin in atopic patients. II. Adverse effects**. J Dermatolog Treat 10: 171-175. The management of atopic dermatitis includes moisturizing creams to reduce the dryness. The adverse skin reactions during topical treatment with two medicinal moisturizers were monitored in a double-blind randomized study on two parallel groups of patients with dry, eczematous skin. One cream contained 4% urea and 4% sodium chloride as active ingredients (23 patients), and the other 5% urea (25 patients). The patients were asked to apply the cream at least once daily for 30 days. The cream containing urea and salt induced skin sensations in about 60% of the patients. Significantly fewer patients experienced sensations with the 5% urea cream. Interestingly, no correlation was found between the severity of the dry skin condition and the degree of smarting. The degree of smarting did not change from day 15 to day 31. The face was reported by the patients to be most sensitive area and five patients (four in one group and one in the other) discontinued or reduced treatment of that area.

Mandy SH. (1974) **Letter: Contact dermatitis to substituted imidazolidinyl urea--a common preservative in cosmetics**. Arch Dermatol 110: 463.

Marsh, GM; Gula, MJ; Youk, AO; et al. (2002) **Bladder cancer among chemical workers exposed to nitrogen products and other substances**. Am J Ind Med 42: 286-295. OBJECTIVE: To investigate further bladder cancer mortality excess based on four deaths observed among the internal comparison population of a previous historical cohort study of workers from a chemical plant in Lima, Ohio. The internal population mainly comprised workers from the Nitrogen Products (NP) Division. METHODS: The original Lima cohort was expanded to include all workers (n = 1,841) employed between 1955 and

1996. A subcohort of workers employed mainly in the NP Division (NP Subcohort) was identified and used as the primary study population and as the source of cases and controls for a matched case-control study of bladder cancer. All death records were reviewed for any mention of bladder cancer; all available personnel, medical, and insurance files were reviewed to identify any current or former employees with an existing diagnosis of bladder cancer. A qualitative exposure assessment of all cases and controls was performed to evaluate an exploratory hypothesis that the bladder cancer excess may be associated with work histories involving both NP and urea, specifically, nitric acid and urea. RESULTS: We identified 96 new deaths among the expanded Lima cohort and obtained cause of death for 90. One additional bladder cancer death and one living case were identified and these were members of the original Lima cohort. The bladder cancer standardized mortality ratio (SMR) for the NP Subcohort (SMR = 3.31, 95%CI = 0.90-8.47) fell between the SMRs observed for all workers in the original and expanded Lima cohort (SMR = 3.93, 95%CI = 1.07-10.06 and 3.10, 95%CI = 1.01-7.24, respectively), but was markedly less than the SMR observed for corresponding subcohort of AN-unexposed workers in the original Lima cohort (SMR = 7.01, 95%CI = 1.91-17.96). Sparse data and a high prevalence of exposure among controls precluded an informative statistical analysis of the possible association between work histories involving both nitric acid and urea and bladder cancer risk. CONCLUSIONS: This study confirms that bladder cancer mortality is elevated among persons who worked mainly in the NP Division of the Lima plant, but at a lower, less statistically significant level than indicated by the original cohort study. No occupational risk factors considered to be causally related to the bladder cancer excess could be identified by this intensive investigation; other possible reasons for the excess are suggested by the descriptive data.

Matthews DE; Downey RS. (1984) **Measurement of urea kinetics in humans: a validation of stable isotope tracer methods**. Am J Physiol 246: E519-27. To define the importance of an accurate priming dose for measurement of urea production using a primed, short-duration (e.g., 4 h) infusion of labeled urea, [18O]- and [13C]ureas were infused simultaneously at two different prime-to-infusion (P/I) doses into five young adult men. The measured mean (+/-SE) urea production rates were 261 +/- 12 and 509 +/- 25 mumol X kg-1 X h-1 for P/I = 12.6-h and 5.0-h priming doses, respectively. In a second series of studies, a single dose of [18O] urea tracer was administered intravenously to four subjects, and the urea production rate was determined from the plasma urea tracer disappearance curve obtained over the following 6 h by fitting the data to a two-exponent curve. The mean urea production rate was 224 +/- 14 mumol X kg-1 X h-1. Because the fractional turnover of the body urea pool is slow, the priming dose strongly influences the "apparent" plasma urea enrichment plateau and, therefore, the measured urea production rate during short-duration infusions. Alternatively, the single-dose protocol can be applied to measure human urea production accurately in periods as short as 6 h.

McClelland I; Danielsen M; Jackson A A. (1992) **Urea Kinetics in Free-Living Adults Measured with a Single-Dose Method**. Proceedings of the Nutrition Society 51: 27-30.

McClelland IS; Jackson AA. (1996) **Urea kinetics in healthy young women: minimal effect of stage of menstrual cycle, contraceptive pill and protein intake**. Br J Nutr 76: 199-209. Urea kinetics were measured using prime/intermittent oral doses of [15N15N]urea, on five separate protocols in thirteen normal young women. Each woman underwent either two or three study protocols. Measurements were made at day 12 and day 22 of the menstrual cycle, whilst consuming their habitual protein intake in seven women not taking the contraceptive pill and in six women taking the contraceptive pill. In three women taking the pill, and three not taking the pill, urea kinetics were measured whilst taking a diet in which the intake was restricted to 55 g protein/d. There was no difference in the rate of urea production, urea excretion or urea hydrolysis between the women taking the pill and those not taking the pill at day 22. In the women not taking the pill there was no difference in any measure between day 12 and day 22. In the

women taking the pill there was a significant difference in the disposal of urea N to excretion or hydrolysis on day 12 compared with day 22, with a relative decrease in excretion and enhancement of hydrolysis at day 12 compared with day 22. On the restricted diet, an intake of 55 g protein/d represented 77% of the habitual intake and urea production, excretion and hydrolysis were reduced to about 84% of the rate found on the habitual intake. In paired studies the reduction in urea production was statistically significant, and there was a statistically significant linear relationship between urea production and either intake or the sum of intake plus hydrolysis. The within-individual variability for urea production was about 10%, for excretion 15% and for hydrolysis 44%. The between-individual variability for intake was about 17% on the habitual intake. The variability for production, excretion and hydrolysis (14, 13, 36%) was less in the women not taking the contraceptive pill than in those taking the pill 23, 32, 42% respectively). The variability was reduced on the controlled low intake of 55 g protein compared with the habitual intake. These results confirm the wide variability in aspects of urea kinetics between individuals. In women this variability is not, to any large extent, accounted for by changes associated with the menstrual cycle. In England, nutritionists measured urea kinetics in 13 women aged 21-37 years who took prime/intermittent oral doses of [15N15N]urea under five separate conditions to identify the extent to which the stage of the menstrual cycle and the use of a low- dose estrogen oral contraceptive (OC) contribute to variability. The protocols included habitual diet alone and urea measurement on either day 12 or day 22 of the menstrual cycle, habitual diet and OC use with urea measurement on either day 12 or day 22, a diet of 55 g protein/day (around 77% of habitual intake) with no control over day of urea measurement, and a diet of 55 g protein/day and OC use with no control over day of urea measurement. The habitual diet had little effect on urea kinetics of the time of the menstrual cycle. OC use also had little effect, except it did decrease excretion (107 vs. 132 mg N/kg/day for non-use) and increase hydrolysis (97 vs. 62 mg N/kg/day) of urea at day 12 of the menstrual cycle. The 55 g/day protein intake decreased urea production, excretion, and hydrolysis (about 84% of habitual diet). It effected the least variation among individuals. For all protocols, the variation in plateau enrichment for urea was 11.8%. The within-individual variability stood at around 10% for urea production, 15% for excretion, and 44% for hydrolysis. For intake, the between-individual variability was around 17% on habitual intake. Nonusers exhibited less variability for production, excretion, and hydrolysis than OC users. In the paired studies, the reduction in urea production and the linear relationship between urea production and either intake or the sum of intake plus hydrolysis was statistically significant. These findings show that healthy young women have urea kinetics similar to those of men and that there is wide variability in urea kinetics between i

McCurdy PR; Mahmood L. (1971) **Intravenous urea treatment of the painful crisis of sickle-cell disease. A preliminary report**. N Engl J Med 285: 992-994.

Meakins TS; Jackson AA. (1979) **Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets**. Clin Sci 90: 215-225. 1. Urea kinetics were measured in six healthy men using prime/intermittent oral doses of [15N15N] urea, after five days consuming one of four diets which varied in their nitrogen content: a reference diet (REF, 70 g of protein and 11.2 g of N); a low-protein diet (LP, 30 g of protein and 4.8 g of N); a low-protein diet with 6.9 g of urea added (LP-U1, 30 g of protein and 8 g of N); a low-protein diet with 13.7 g of urea added (LP-U2, 30 g of protein and 11.2 g of N). 2. Apparent nitrogen balance on the REF diet was significantly better than on the LP or the LP-U1 diets. The addition of the higher level of urea in the LP-U2 diet enhanced apparent nitrogen balance compared with the LP or LP-U1 diets, and was not different to apparent nitrogen balance on the REF diet. 3. On the LP, LP-U1 and LP-U2 diets, the rate of endogenous urea production was not different, and was about 60% of that on the REF diet, a statistically significant difference. The addition of a dietary supplement of urea increased the rate of urea appearance in the urea pool in direct relation to the dose of urea taken. There was no difference in the rate of appearance

between the REF and LP-U2 diets, for both of which the rate of appearance was significantly greater than on the LP diet. 4. The excretion of urea in urine on the LP diet was 62% of that on the REF diet, a significant difference. There was no significant difference in the rate of urea excretion between the REF, LP-U1 and LP-U2 diets. 5. The rate of urea hydrolysis by the colonic microflora on the REF diet was more than twice that on the LP or LP-U1 diets. Supplementation with urea at the higher level, LP-U2, significantly increased hydrolysis to the same level as on the REF diet. Most of the nitrogen derived from urea hydrolysis was retained in the metabolic pool (> 80%), with no difference in the rate of retention between the REF and LP-U2 diets, both greater than the LP or LP-U1 diets. 6. The dietary supplements of urea increased the size of the body urea pool significantly. Renal clearance of urea was highest on the REF diet and decreased 13-29% on the low-protein diets. Bowel clearance was highest on the REF diet and decreased 46-55% on the low-protein diets. Neither urinary excretion of urea nor urea hydrolysis in the bowel were related simply to the concentration of urea in blood. Urea hydrolysis related most closely to the rate of appearance of urea in the urea pool. 7. The salvage of urea nitrogen was increased on the highest level of supplementation, but the overall sensitivity of the system was low, suggesting that other factors might be limiting for effective urea hydrolysis and the salvage of urea nitrogen.

Meakins TS; Persaud C; Jackson AA. (1998) **Dietary supplementation with L-methionine impairs the utilization of urea-nitrogen and increases 5-L-oxoprolinuria in normal women consuming a low protein diet**. J Nutr 128: 720-727. Urea kinetics were measured in normal women after 5 d consuming a low protein diet [LP, 67 mg N/(kg.d), 0.42 g protein/(kg.d)]. To determine whether the availability of methionine limits the utilization of nonessential nitrogen from low protein diets, the study was repeated on four further occasions with the addition of dietary supplements of L-methionine, 9 mg N/(kg.d) (LP-M); urea, 52 mg N/(kg.d) (LP-U); urea and methionine (LP-UM); or urea, 26 mg N/(kg.d), and glycine, 26 mg N/(kg.d), (LP-UG). Urea kinetics were derived after prime and intermittent oral doses of [15N15N] urea from the measurements of enrichment by isotope ratio mass spectrometry in urea isolated from urine. Nitrogen balance was significantly improved when the women consumed LP-U and LP-UG, but not LP-M or LP-UM. The urinary excretion of 5-L-oxoproline was measured as a marker of glycine availability and was significantly lower when women consumed LP-U and LP-UG compared with either LP or LP-M and LP-UM. There was a significant correlation between urinary 5-L-oxoproline and urinary sulfate excretion ($r = 0.68$, $P = 0.00003$). The availability of methionine was not limiting for nitrogen metabolism when women consumed these diets, whereas the response to supplementation with urea alone or urea with glycine showed that the availability of nonessential nitrogen was limiting. Glycine is consumed in the detoxification of excess methionine, and supplementation with methionine appeared to place a competitive demand on the availability of glycine for other metabolic processes.

Metges CC; Petzke KJ; El-Khoury AE; et al. (1999) **Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates**. Am J Clin Nutr 70: 1046-1058. BACKGROUND: The importance of urea nitrogen reutilization in the amino acid economy of the host remains to be clarified. OBJECTIVE: The objective was to explore the transfer of (15)N from orally administered [(15)N(2)]urea or (15)NH(4)Cl to plasma free and intestinal microbial amino acids. DESIGN: Six men received an L-amino acid diet (167 mg N*kg(-)(1)*d(-)(1); 186 kJ*kg(-)(1)*d(-)(1)) for 11 d each on 2 different occasions. For the last 6 d they ingested [(15)N(2)] urea or, in random order, (15)NH(4)Cl (3.45 mg (15)N*kg(-)(1)*d(-)(1)). On day 10, a 24-h tracer protocol (12 h fasted/12 h fed) was conducted with subjects receiving the (15)N tracer hourly. In a similar experiment, (15)NH(4)Cl (3.9 mg (15)N*kg(-)(1)*d(-)(1)) was given to 7 ileostomates. (15)N Enrichments of urinary urea and plasma free and fecal or ileal microbial protein amino acids were analyzed. RESULTS: (15)N Retention was significantly higher with (15)NH(4)Cl (47.7%; $P < 0.01$) than with [(15)N(2)]urea (29.6%). Plasma dispensable amino acids after the (15)NH(4)Cl tracer were enriched up to 20 times (0. 2-0.6 (15)N atom% excess) that achieved with [(15)N(2)]urea. The (15)N-labeling pattern of plasma,

ileal, and fecal microbial amino acids (0.05-0.45 (15)N atom% excess) was similar. Appearance of microbial threonine in plasma was similar for normal subjects (0.14) and ileostomates (0.17). CONCLUSION: The fate of (15)N from urea and NH(4)Cl differs in terms of endogenous amino acid metabolism, but is similar in relation to microbial protein metabolism. Microbial threonine of normal and ileostomy subjects appears in the blood plasma but the net contribution to the body threonine economy cannot be estimated reliably from the present data.

Mikhailuts AP; Artamonova GV. (1989) **Hygienic assessment of work capacity of workers retiring on a pension in nitrogen compound manufacturing plants**. Gig Tr Prof Zabol 3: 4-7. Workers retiring early due to hazardous work conditions in the manufacture of NH3, HNO3, NH4NO3, and urea experienced biol. stress and overstress of the major body systems, decrease of muscular and mental capacity both at the beginning and during the work shift, and increase in chronic morbidity and temporary disability rates. These indicators were not identical in groups with different working conditions and sex. Among machine operators, the majority of males before retirement (vs. females) maintained a more satisfactory level of work capacity. Disease rates and the degree of decrease of functional body capacities were higher in male metal workers in comparison with machine operators.

Monge C; Torres C; Ramirez M. (1961) **Interrelationships between serum creatinine, urea, sulfate and endogenous creatinine clearance in man**. Acta Physiol Lat Am 11: 4-9.

Moran BJ; Jackson AA. (1979) **Metabolism of 15N-labelled urea in the functioning and defunctioned human colon**. Clin Sci 79: 253-258. 1. The luminal metabolism of urea was studied using double-labelled urea ([15N2]urea) which was placed in the lumen of the colon through a colostomy. The recovery of label was measured as [15N2]urea or [14N, 15N]urea in urine and as 15N in stool. 2. Five patients with a loop colostomy allowed a comparison of the right functioning colon with the left defunctioned colon in the same individual. Five subjects with a left end-colostomy enabled a comparison of the right with the left functioning colon. 3. A significantly greater proportion of labelled urea was recovered as [15N2]urea in the urine when the dose was placed in the left defunctioned colon (29%) compared with either the left or the right functioning colon (9 and 4%, respectively). This is interpreted as being a result of a decrease in the bacterial activity and concomitant urea hydrolysis in the defunctioned colon. 4. On average more than half of the label was retained in the body, regardless of whether the urea was placed in the functioning or the defunctioned colon, on the left or on the right. 5. The data confirm that the colon is permeable to the intact urea molecule. Intraluminal urea is readily hydrolysed in the functioning colon. A large proportion of the nitrogen released by urea hydrolysis may be retained within the metabolic nitrogen pool of the host. There are significant differences in the handling of urea nitrogen in the defunctioned colon relative to the functioning colon.

Moran BJ; Jackson AA. (1990) **15N-urea metabolism in the functioning human colon: luminal hydrolysis and mucosal permeability**. Gut 31: 454-457. The biopsy channel of the colonoscope was used in a novel approach to the study of in vivo colonic nitrogen metabolism in 12 subjects. A tracer dose of 15N15N-urea was placed in the caecum in six and distal to the splenic flexure in six. The urine and stool were collected for 72 hours and isotopic enrichment was measured in a mass spectrometer. A similar proportion of the dose was recovered in the urine as 15N15N-urea from the right colon, 6%, as was recovered from the left, 4%, showing that the urea was absorbed intact. Urinary 15N14N-urea from the right colon was 18% of the dose compared to 13% from the left colon. This represents urea that has been hydrolysed and absorbed as ammonia. Less than 4% of the dose was recovered in the stool. The greatest proportion of the label, 74% from the right and 82% from the left, could not be accounted for in the urine or the stool and is presumed to have entered the metabolic pool of nitrogen. We conclude that; the colon is permeable to urea, intraluminal hydrolysis occurs and that urea nitrogen enters the metabolic

pool of nitrogen in functionally significant quantities.

Mukarram ABM; Habibullah CM; Swamy M; et al. (1992) **Studies on urea cycle enzyme levels in the human fetal liver at different gestational ages**. Pediatr Res 31: 143-145. Urea cycle enzymes involved in the detoxification of ammonia were studied in liver tissues of 57 male and 49 female fetuses of different age groups ranging from 13 to 36 wk of gestation. Surgical wedge biopsies of liver from 18 male and 12 female adults were used as controls. Significant enzyme activity was found to be present as early as the 13th wk of gestation. As gestational age advanced, enzyme activity gradually increased, reaching about 90% of the adult activity by the 36th wk of gestation.

Muranda, M; Etcheverry, R; Becker, P; et al. (1972) **[Therapeutic evaluation of oral urea in a case of sickle cell anemia]**. Rev Med Chil 100: 426-429.

Nalbandian, RM; Shultz, G; Lusher, JM; et al. (1971) **Sickle cell crisis terminated by intravenous urea in sugar solutions--a preliminary report**. Am J Med Sci 261: 309-324.

Not Given. (1973) **Urea for treatment of dry skin**. Med Lett Drugs Ther 15: 104.

Not Given. (1980) **Topical corticoid therapy: a round table discussion. Part IV. The proper role of urea in managing skin disease**. Cutis 25: 318-319.

Not Given. (1987) **High plasma urea concentrations in collodion babies**. Arch Dis Child 62: 212.

Opio E; Barnes PM. (1972) **Intravenous urea in treatment of bone-pain crises of sickle-cell disease. A double-blind trial**. Lancet 2: 160-162.

Parmley TH; Burnett LS; Blake DA; et al. (1976) **The possible deleterious effects of the intramyometrial injection of hypertonic urea**. Obstet Gynecol 47: 210-212. Hypertonic urea has been shown to be an effective midtrimester abortifacient. Although safer than hypertonic saline when injected intravascularly, it has not been compared to saline in the case of inadvertent intramyometrial injection. This report documents that intramyometrial injection of hypertonic urea will result in the same type of muscle necrosis as that produced by saline.

Passaro G; Barbati M. (1950) **[Blood urea clearance in children under normal conditions and in kidney diseases]. LA "BLOOD UREA CLEARANCE" NEI BAMBINI IN CONDIZIONI NORMALI E NELLE NEFROPATIE**. Arch Ital Pediatr Pueric 14: 135-153.

Patterson BW; Carraro F; Klein S; et al. (1995) **Quantification of incorporation of [15N]ammonia into plasma amino acids and urea**. Am J Physiol 269: E508-15. The incorporation of 15N into individual plasma amino acids and urea was quantified in five human subjects who received 15NH4Cl either orally or intravenously for 6 h. After oral tracer administration, the highest enrichment was achieved by arginine, followed by urea and glutamine; distribution of 15N within glutamine was 55% amide and 45% amino N. Glutamine achieved the highest enrichment after the intravenous administration of tracer, with a distribution of 92% amide and 8% amino N. The relative distribution pattern of 15N incorporation was quantified from the rate at which 15N initially appeared in each plasma component. Amino acids (especially arginine, glutamine, and glutamate) accounted for greater than one-half (54%) of the orally administered tracer that was initially recovered in plasma components, compared with 46% initial appearance for urea; for the intravenous tracer, amino acids accounted for 78% of initial appearance of tracer compared with 22% for urea. Our results highlight the involvement of the splanchnic bed in the

utilization of orally administered ammonia (preferential incorporation of oral tracer into arginine, urea, glutamate, and the amino N of glutamine) in contrast to the preferential incorporation of systemically administered ammonia into the amide N of glutamine and alanine.

Pokorny J. (1967) **[Urea during resuscitation of neurological patients]**. Cesk Neurol 30: 342-343.

Rajka E. (1962) **[Experimental studies on the mechanism of formation of urea blisters. I. Effect of O2 supply on the formation of urea blisters on the human skin.]**. Hautarzt 13: 248-253.

Ranade, K; Wu, KD; Hwu, CM; et al. (2001) **Genetic variation in the human urea transporter-2 is associated with variation in blood pressure**. Hum Mol Genet 10: 2157-2164. The kidney, by regulating the volume of fluid in the body, plays a key role in regulating blood pressure (BP). The kidney uses primarily sodium and, to a lesser extent, urea to maintain the appropriate volume of fluid. Genetic variation in proteins that determine sodium reabsorption and excretion is known to significantly influence BP. However, the influence of genetic variation in urea transporters on BP has not been examined. We determined therefore whether nucleotide variation in the kidney-specific human urea transporter, HUT2, is associated with variation in BP. After determining the genomic structure of the coding sequence, seven single nucleotide polymorphisms (SNPs) were identified. Two of the SNPs result in Val/Ile and Ala/Thr amino acid substitutions at positions 227 and 357 in the HUT2 open reading frame, respectively. Another SNP is silent and four others are in introns or the 3' untranslated region. Over 1000 hypertensive and low-normotensive individuals of Chinese origin were typed for five of these SNPs using a high-throughput genotyping method. The Ile227 and Ala357 alleles were associated with low diastolic BP in men but not women, with odds ratios 2.1 [95% confidence interval (CI) 1.5-2.7, $P < 0.001$] and 1.5 (95% CI 1.2-1.8, $P < 0.001$), respectively. There was a similar trend for systolic BP, and odds ratios for the Ile227 and Ala357 alleles were 1.7 (95% CI 1.2-2.3, $P = 0.002$) and 1.3 (95% CI 1.1-1.6, $P = 0.007$), respectively, in men.

Ravin MB; Garber V; Gibson EL. (1964) **intravascular hemolysis following urea administration during hypothermia**. Anesthesiology 25: 576-580.

Rumyantsev, GI; Kozlova, TA; Atyakina, IK; et al. (1980) **Working conditions and health status of workers in the manufacture of urea**. Gig Sanit: 17-19. Results of studies of the occupational environment and of physiologic and hygienic characteristics of working conditions at the various stages of urea manufacturing are presented. General morbidity rates of workers involving temporary working incapacity for the period 1976-1978 are analyzed.

Rupa, DS; Rita, P; Reddy, PP; et al. (1988) **Screening of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes of vegetable garden workers**. Hum Toxicol 7: 333-336. 1. Twenty-five male workers occupationally exposed to DDT, BHC malathion, parathion, dimethoate, fenitrothion, urea and gromor were selected as subjects for the analysis of chromosomal aberrations and sister chromatid exchanges (SCE) in peripheral lymphocytes. 2. Blood samples were collected from 30 normal healthy males from the same age group and socioeconomic class for the control. 3. The frequency of chromosomal aberrations and SCEs increased significantly irrespective of the duration of exposure to pesticides, when compared to controls.

Rutkowski, P. (2006) **[Clinical and metabolic consequences of uremic toxicity]**. Przegl Lek 63: 209-217. Retention of many substances takes place in the pathogenesis of uremic toxicity. There are almost 100 different molecules described and defined as uremic toxins. These substances are divided into three groups according to EUTOX group calssification. Small water soluble molecules with a molecular

weight less than 500 D are included into the first group. Derivate of guanidines, purines, pyrimidines and methyloamines appeared in this group. There is also an unclassified subgroup with urea as a "classical" toxin which the real role in the uraemic syndrome is still discussed. Main symptoms caused by these molecules are digestive disturbances, neurological changes, hypertension etc. We can eliminate almost all of these toxins with standard methods used during dialysotherapy. Substances with a different molecular weight but connected with proteins determine the second group. AGE-s, phenol derivates, leptin and poliamines beside others create this group. There are many studies that have proved that these toxins cause hypertension, arteriosclerosis and shortened life time of hemodialysed patients. However, melatonin toxicity is not fully proved. Different types of renal replacement therapy are not valid to purify blood from protein-bound substances. Middle molecules are included into the third group, with a molecular weight higher than 500 D. There are cytokines, neuro-transmitters e.g. beta-endorphin, metencephalin and many others accounted into this group. One of them is the parathormon, well known and considered as "universal" toxin for several years. Middle molecules are causing very different effects. They are responsible for: anemia, arteriosclerosis, chronic inflammation and generally increase dialysed patient mortality. Toxic action of several molecules described below is still not proved; however there are some ongoing studies aimed to find pathophysiological links between old and new described uremic toxins.

Rypins E; Henderson JM; Rudman D; et al. (1979) **A pharmaco kinetic method for measuring urea synthesis in normal and cirrhotic subjects**. Gastroenterology 77: A36.

Rypins EB; Henderson JM; Fulenwider JT; et al. (1979) **Pharmacokinetic method for measuring urea synthesis rates**. Surg Forum 30: 390-392.

Rypins EB; Henderson JM; Fulenwider JT; et al. (1980) **A tracer method for measuring rate of urea synthesis in normal and cirrhotic subjects**. Gastroenterology 78: 1419-1424.

San Pietro A; Rittenberg D. (1953) **A study of the rate of protein synthesis in humans. I. Measurement of the urea pool and urea space**. J Biol Chem 201: 445-455.

San Pietro, AG. (1954) **A study of the rate of protein synthesis in humans. I. Measurement of the urea pool and urea space. II. Measurement of the metabolic pool and the rate of protein synthesis**. Diss Abstr 14: 13.

Schubert H; Wurbach G; Godenschweger K. (1984) **[Effectiveness of hand-protective salves in urea contamination]**. Z Gesamte Hyg 30: 523-525.

Shannon IL; Prigmore JR. (1961) **Effects of urea dosage on urea correlations in human parotid fluid and blood serum**. Arch Oral Biol 5: 161-167.

Shaw JH; Klein S; Wolfe RR. (1985) **Assessment of alanine, urea, and glucose interrelationships in normal subjects and in patients with sepsis with stable isotopic tracers**. Surgery 97: 557-568. The kinetic interactions among glucose, alanine, and urea metabolism were studied in both normal volunteers and in patients with sepsis by means of a primed, constant infusion of stable isotopes. In the normal volunteers, infusion of glucose at 4 mg /kg/min suppressed total glucose production, the rate of gluconeogenesis from alanine, and the production of urea, despite an increase in the rate of release and uptake of alanine. When the glucose infusion rate was increased to 8 mg/kg/min, the production of urea decreased further, even though gluconeogenesis from alanine was already suppressed by the first infusion. This additional N-sparing effect was explainable by an increase in glucose oxidation. In the

patients with sepsis the basal rates of production of glucose and urea were elevated significantly. Glucose infusion (4 mg/kg/min) decreased hepatic glycogenolysis but not gluconeogenesis from alanine or urea production. At the glucose infusion rate of 8 mg/kg/min, glucose oxidation increased in the patients and urea production decreased. Thus in patients with sepsis a higher rate of glucose infusion is necessary to achieve nitrogen-sparing effects than is necessary in controls because of a lack of suppressibility of gluconeogenesis. Because of continued glucose production during glucose infusion, hyperglycemia commonly develops during glucose infusion in sepsis. However, this effect does not necessarily indicate a complete inability of the patient with sepsis to benefit nutritionally from infused glucose, as we observed no decrement in the ability to oxidize infused glucose.

Stein J; Krejcova H. (1969) **The influence of intravenous infusion of hypertonic urea on the EEG of patients with intracranial space-occupying processes**. Electroencephalogr Clin Neurophysiol 26: 228.

Stevens C; Kennaway NG; Fellman JH. (1975) **Ammonia intoxication: a hazard during rehabilitation of protein-deprived rats [Malnutrition, urea cycle]**. J Nutr 105: 1384-1390.

Steyn, DG. (1961) **An outbreak of urea poisoning among Bantu farm laborers in the Potgietersrust district, Transvaal**. S Afr Med J 35: 721-722. An outbreak of urea poisoning in Bantu farm laborers as a result of mistaking Kynoch's Urea Fertiliser for common salt is described. ABSTRACT AUTHORS: Author

Tarantino C; Pasquinelli F. (1949) **Urea synthesis in human placenta. Original Title: LA SINTESI DELL'UREA NELLA PLACENTA UMANA**. Sperimentale 99: 435-443. Human placenta at term can synthesize urea from NH4CI, but not up to the 7th month of pregnancy. ABSTRACT AUTHORS: Claudio Gerbi

Thomas RD; Newill A; Morgan DB. (1979) **The cause of the raised plasma urea of acute heart failure**. Postgrad Med J 55: 10-14.

Tizianello A; de Ferrari G; Garibotto G; et al. (1978) **Cerebral and hepatic urea synthesis in patients with chronic renal insufficiency**. Proc Eur Dial Transplant Assoc 15: 500-505. Urea production by the liver and the brain was evaluated in patients with chronic renal insufficiency and in subjects with normal renal function by measuring the arterial-venous differences of urea across the hepatosplanchnic bed and the brain. In five out of seven patients with chronic renal insufficiency no urea release into the hepatic veins was observed, whereas a high urea output by the brain was measured in 6 out of 8 patients. In the control group urea was released only into the hepatic veins. These data demonstrate a defect in hepatic urea synthesis and a switch to cerebral ureagenesis in chronic renal insufficiency.

Ummenhofer B; Djawari D. (1979) **[Cross allergy between sulphonamide diuretics, probenecid, sulphamethoxazole and sulphonyl-urea compounds (author's transl)]**. Dtsch Med Wochenschr 104: 514-517. Typical signs and symptoms of type III allergy occurred in a 55-year-old diabetic during diabetes and diuretic treatment with glisoxepid, glibenclamide, furosemide and probenecid. Symptoms of this type of allergy unintentionally recurred with every subsequent therapeutic administration of each one of these drugs. The possibility of a cross allergy between sulphonamide diuretics, chemotherapeutic sulphonamides and sulphonyl-urea compounds has only rarely been described.

Van Slyke DD; Alving A; Rose WC. (1932) **Studies of urea excretion. VII. The effects of posture and exercise on urea excretion**. J Clin Invest 11: 1053-1064. In subjects with more than 50% renal function measured by the blood urea clearance, the clearance is essentially the same, with the subject kept in bed

or walking about. In 3 out of 12 nephritics with less than 50% normal clearance, walking depressed the clearance markedly, the mean values being 44, 60 and 69% of the values with patients in bed. Severe exercise in 3 normal subjects depressed the clearance somewhat, but only in 3 out of 22 were clearances below 70% of the mean normal value. Thus, in subjects with clearance values over 50% of the average normal, the clearance can be determined with subjects ambulatory, while in those below 50% it is essential to keep them at rest during the 2-hr. excretion period. ABSTRACT AUTHORS: A. Alving

Vilstrup H; Hansen BA; Almdal TP. (1990) **Glucagon increases hepatic efficacy for urea synthesis**. J Hepatol 10: 46-50. The effect of glucagon on the relation between urea synthesis and blood amino acid concentration was studied in seven healthy volunteers. Alanine was given as prime-continuous infusions and, after 1 hr for equilibration, the urea nitrogen synthesis rate was measured in two periods of about 2 hrs as urinary excretion corrected for accumulation and intestinal hydrolysis. During one of the periods, glucagon was infused to obtain a constant concentration of 200-1200 ng/l. The spontaneous urea synthesis during the alanine infusion was 86-141 mmol/hr and linearly related to the alanine concentrations of 1.33-2.99 mmol/l. The hepatic clearance of alanine-nitrogen to urea-nitrogen, assessed by the ratio between the increase in the urea synthesis rate and alanine concentration, was 23 +/- 4 l/hr (mean +/- S.D.). Glucagon increased the rate of urea synthesis by 35 +/- 11 mmol/hr (p less than 0.02) and decreased the alanine concentration by 0.22 +/- 0.06 mmol/l (p less than 0.01). Glucagon increased the hepatic nitrogen clearance to an average of 42 +/- 13 l/hr (p less than 0.01). The difference between infusion of amino-nitrogen and appearance of urea-nitrogen was +15 +/- 10 mmol/hr during alanine infusion alone and -11 +/- 25 mmol/hr during exogenous glucagon. The loss of nitrogen could be accounted for by depletion of non-alanine amino acids from the blood. Glucagon increases the efficacy of urea synthesis, which may be of importance for catabolism by changing the hepatic contribution to nitrogen homeostasis.

Weijs PJ; Calder AG; Milne E; et al. (1996) **Conversion of [15N]ammonia into urea and amino acids in humans and the effect of nutritional status**. Br J Nutr 76: 491-499. Hepatic NH3 detoxification by ureagenesis requires an input of aspartate-N, originating either from amino acid-N or NH3-N. The relative importance of these two routes may depend on the nutritional state. To test this, four volunteers were given a liquid diet for 2 d and then on day 3 were either fed every 20 min or fasted. Doses of 15NH4Cl were taken orally every 20 min for 6 h (total 1.5 g) and blood was sampled hourly. Urea-N elimination under fasted conditions was only 0.75 of that for the fed state. Considering the increase in body urea pool during feeding, ureagenesis during fasting was probably closer to 0.6 of that during feeding. Since the [14N15N]urea enrichment was not different between the fed and fasted states, the proportion of the 15NH3 dose converted to urea during fasting was also 0.6 of that during the fed condition. No change in [14N15N]urea and [amide-15N]glutamine enrichment suggested that NH3 enrichment was also not affected by nutritional state. Enrichment of [15N15N]urea was approximately 0.05 that of [14N15N]urea which indicates that 15NH3 can also enter the aspartate route, the importance of which is yet unknown. Both [15N15N]urea and [amino-15N]glutamine enrichment in the fasted state were approximately 1.7 times that in the fed state, indicating increased labelling of precursors and/or increased NH3 flux through the aspartate route. Glutamate, valine, leucine and isoleucine showed comparable increases in enrichment during fasting. Arginine enrichment was unaltered by nutritional state, but was lower than [14N15N]urea, indicating incomplete equilibration with the arginine pool in periportal hepatocytes. The present study indicates that hepatic NH3 detoxification may use the aspartate route, gaining importance in the fasted state. The majority of urea was supplied with only one N atom from NH3, thus provision of the other may have consequences for alternative substrates, in particular amino acids.

Wicentowicz Z. (1967) **[Studies on the action of urea in neurosurgical cases]**. Folia Med Cracov 9:

29-65.

Wolfe RR; Wolfe MH; Nadel ER; et al. (1984) **Isotopic determination of amino acid-urea interactions in exercise in humans**. J Appl Physiol 56: 221-229. We recently reported that in light exercise (30% VO2max) the oxidation of [1-13C]leucine was significantly increased but the rate of urea production was unchanged (J. Appl. Physiol: Respirat. Environ. Exercise Physiol. 52: 458-466, 1982). We have therefore tested three possible explanations for this apparent incongruity. 1) We infused NaH13CO3 throughout rest and exercise and found that, although altered bicarbonate kinetics in exercise resulted in greater recovery of 13CO2, the difference between rest and recovery was small compared with the increase in the rate of 13CO2 excretion during exercise when [1-13C]leucine was infused. 2) We infused [15N]leucine and isolated plasma urea N to determine directly the rate of incorporation of the 15N. During exercise there was no increase in the rate of 15N incorporation. Simultaneously, we infused [2,3-13C]alanine and quantified the rate of incorporation of 15N in alanine. We found that [15N]alanine production from [15N] leucine more than doubled in exercise, and by deduction, alanine production from other amino acids also doubled. 3) We tested our previous assumption that [1-13C]leucine metabolism in exercise was representative of the metabolism of other essential amino acids by infusing [1-13C] and [alpha-15N]lysine throughout rest and exercise. We found that the rate of breakdown of lysine during exercise was not increased in a manner comparable to that of leucine. Thus, these data confirm our original findings that leucine decarboxylation is enhanced in light exercise but urea production is unchanged.(ABSTRACT TRUNCATED AT 250 WORDS)

Wrong OM; Vince AJ; Waterlow JC. (1985) **The contribution of endogenous urea to faecal ammonia in man, determined by 15N labelling of plasma urea**. Clin Sci 68: 193-199. The plasma urea of 2 healthy men was labelled with 15N at a constant rate for several days and its 15N enrichment was compared with that of faecal ammonia and total nitrogen. Faeces collected after one complete gastrointestinal transit from the onset of plasma labelling had ammonia 15N enrichments which were only 8.5 [plusmn] 1.2% and total N enrichments which were 6.8 [plusmn] 0.7% of the plasma urea 15N enrichment. Results show that endogenous urea is not the main precursor of faecal ammonia, which is probably derived by bacterial deamination from the protein of dietary residues, intestinal secretions and shed epithelial cells. The minor contribution of endogenous urea to faecal ammonia suggests that the lumen of the large bowel is not the main site of endogenous urea hydrolysis. The similar labelling of faecal total N and ammonia N supports other evidence that these faecal N fractions are in a constant state of exchange.

Young MM. (1982) **Treatment of chronic plaque psoriasis with dithranol and urea creams: a double-blind study**. Pharmatherapeutica 3: 86-92. Stable cream formulations containing 0.1% or 0.2% dithranol in a 17% urea base have been developed. A double-blind comparative trial in 20 out-patients showed that over a 6-week treatment period the 0.2% dithranol and urea cream was significantly more effective (p less than 0.01) than the 0.1% dithranol and urea cream in clearing chronic plaque psoriasis. The 0.2% dithranol/urea cream was accompanied by a higher incidence of minor, irritant side-effects, but was still well accepted by patients for use at home. The availability of differing strengths of dithranol and urea cream enables practitioners to tailor dithranol treatment in accordance with the individual patient's clinical response

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Balestri P L; Rindi P; Biagini M. (1971) **Chronic urea intoxication in dogs**. Experientia 27: 811-812.

Button, C; Joubert, JP; Maartens, BP. (1982) **Absence of urea toxicity in young pigs**. J S Afr Vet Assoc 53: 67-68. Urea was non-toxic to a 10 week-old pig in an acute dose as high as 16 g/kg body mass. Ten % m/m urea in pig food over a period of 5 days was also without apparent deleterious effect.

Felinski, L. (1957) **[Toxicity of urea in calves.]**. Acta Physiol Pol 8: 320-321.

Finlayson, JS; Baumann, CA. (1956) **Responses of rats to urea and related substances. The use of a spaced-feeding technique**. J Nutrition 59: 211-221. Spaced feeding, the practice of feeding rats for only two hours per day, has been found to increase the growth-depressing action of several nitrogenous compounds. Dietary urea depressed growth in both spaced and orthodox experiments, but 5% of urea fed two hours per day was as effective as 30% fed ad libitum. The depression in growth has been correlated with the rate of urea intake and the level of urea in the blood, and was not affected by the level or adequacy of the dietary protein. Spaced feeding increased the toxicity of L-leucine, diammonium citrate, ammonium carbonate, and 2,4-dinitro-toluene. Growth depressions by the ammonium salts varied directly with blood urea. This regimen lessened the toxicity of 3[image]-methyl-4-dimethylaminoazobenzene and ethanol but had little effect on relative growth rates when biotin, vitamin B12 and folic acid were omitted from the diet or when glycine or antibiotics were added. The procedure shows promise in measuring the biological value of small amounts of protein.

Fleischman, RW; Baker, JR; Hagopian, M; et al. (1980) **Carcinogenesis bioassay of acetamide, hexanamide, adipamide, urea and p-tolylurea in mice and rats**. J Environ Pathol Toxicol 3: 5-6. As a part of the National Cancer Institute's effort to screen environmental and occupational chemicals for chronic toxicity and carcinogenicity, the amides acetamide, hexanamide, adipamide, urea, and p-tolylurea were fed to male and female C57B1/6 mice and Fischer 344 rats from 12 months. Rats received the following concentrations of compounds in their diets: acetamide, 2.36%; hexanamide, 1.5%; adipamide, 2.4% and 5.8%; urea, 0.45%, 0.9%, and 4.5%; and p-tolylurea, 0.2%. Mice received acetamide, 1.18% and 2.36%; hexanamide, 1.0% and 1.5%; adipamide, 1.6% and 2.4%; urea, 0.45%, 0.9%, and 4.5%; and p-tolylurea, 0.2%. In acetamide treated rats, there was a compound-related occurrence of neoplastic nodules (1/47 male, 3/48 female) and hepatocellular carcinomas (41/47 male, 33/48 female). The incidence, speed of onset and frequency of metastases, were greater in males than in females. In male mice treated with acetamide (low dose, 7/50; high dose, 7/46); hexanamide (low dose, 6/35; high dose, 6/39); and p-tolylurea (10/43), there was a compound-related increase in hematopoietic tumors, namely malignant lymphomas. Therefore, under the conditions of these studies, acetamide was an hepatocarcinogen in rats. In male mice, acetamide, hexanamide, and p-tolylurea caused malignant lymphomas. Urea and adipamide were considered to be non-carcinogenic.

Jagos, P; Dvorak, V; Illek, J. (1981) **[The effect of long-term feeding of higher doses of urea on clinical and biochemical indicators in sheep]**. Vet Med 26: 101-107. [Czech] During feeding 120 g of urea per head/day to four-year wethers for nine months it was observed that for the whole period the animals accepted the feed with appetite, the rumen motility and body temperature were within the physiological standard. In both groups of animals (three animals experimental and three animals control) a higher breathing frequency was found. In experimental (control) animals the levels were as follows: plasma calcium 2.62 +/- 0.17 mmol . 1(-1) (2.66 +/- 0.16 mmol . 1(-1)), inorganic phosphorus 2.35 +/-

0.33 mmol . 1(-1) (2.40 +/- 0.47 mmol . 1(-1), sodium 148.25 +/- 5.65 mmol . 1(-1) (151.09 +/- 5.71 mmol . 1(-1), potassium 5.02 +/- 0.46 mmol . 1(-1) (5.02 +/- 0.74 mmol . 1(-1)), magnesium 1.00 +/- 0.14 mmol . 1(-1) (0.98 +/- 0.10 mmol . 1(-1)), chlorine 107.86 +/- 12.86 mmol . 1(-1) (106.92 +/- 11.12 mmol . 1(-1)), total protein 69.9 +/- 7.8 g. 1(-1) (70.2 +/- 8.1 g . 1(-1)), glucose 3.97 +/- 0.68 mmol . 1(-1) (4.48 +/- 1.01 mmol . 1(-1)), urea 11.20 +/- 3.23 mmol . 1(-1) (7.61 +/- 1.89 mmol . 1(-1)), osmotic pressure 299.27 +/- 12.91 mosm . kg-1 (298.63 +/- 10.44 mosm . kg-1). By statistical t-test evaluation a significant difference in plasma urea in favour of the experimental animals was found. As to the other followed values, a statistically significant difference between the two groups, and that in favour of the control animals, was found only as regards the pulse rate.

Javid M; Anderson J. (1958) **Observations on the use of urea in Rhesus Monkeys**. Surgical forum 9: 686-690.

Levine, S; Saltzman, A. (2001) **Are urea and creatinine uremic toxins in the rat?** Ren Fail 23: 53-59. Urea and creatinine are not generally considered to be important uremic toxins despite evidence from dialysis expts. to the contrary, and despite striking elevations of these nitrogenous waste products in uremia. In order to study this problem in acute uremia, we used a new dietary method for prolonging the survival of bilaterally nephrectomized rats. Urea or creatinine were injected on three successive days starting one day after the inception of uremia. Urea or creatinine injections shortened the survival time of acutely uremic rats, and increased the involution of thymus and spleen. The extra urea, but not creatinine, increased the serum osmolality. These data indicate that urea and creatinine are toxic in the acutely uremic rat. Hypertonicity of the serum may contribute to the toxicity of urea. Addnl. mechanisms of toxicity and addnl. toxins are not excluded.

Lutsyuk, NB; Shamrai, PF; Borisenko, BA; et al. (1980) **Experimental study of the effect of small doses of urea on the body of animals under chronic study**. Ratsional'n Pitanie, Kiev: 76-80. Title only translated.

Ortolani, El; Mori, CS; Rodrigues Filho, JA. (2000) **Ammonia toxicity from urea in a Brazilian dairy goat flock**. Vet Hum Toxicol 42: 87-89. A flock of goats received a diet with 1% urea for at least 1 y. A new batch of concentrate was offered increasing the level of urea to 4.2%. Eighteen of 54 goats showed acute signs of ammonia toxicosis. Ten goats died within 60 min; 4 goats and a buck with convulsions recovered when treated by administration of vinegar and infusion of saline solution, diuretics, and atropine. Three goats with mild signs recovered within 1 h without treatment. The mean ammonia concentration and rumen pH content were 820 mg/L and 7.7, respectively. Generalized congestion, intense pulmonary edema, and slight tubular nephrosis were found in 3 goats on necropsy. The outbreak was self-limiting and no more cases occurred when the diet was removed.

Patel, H; Cohen, B. (1980) **Some observations concerning the toxicity of urea in uremia**. Clin Chem 26: 1036-1037.

Raidal, SR; Jaensch, SM. (2006) **Acute poisoning of silver gulls (Larus novaehollandiae) following urea fertilizer spillage**. Avian Pathol 35: 38-41. Two episodes of accidental urea toxicosis are described in wild silver gulls (Larus novaehollandiae) following spillage of fertilizer grade urea at a commercial shipping facility near Perth, Western Australia. In both cases, urea spillage had been seen to contaminate freshwater wash-down pools on the wharves where ships were being unloaded and gulls were seen to be drinking and washing in the pools nearby the spillages. Affected birds were found moribund or dead. Necropsy and histopathological findings were non-specific and consisted of mild to moderate congestion of visceral organs and brain. Analysis of a water sample collected

during Case 1 revealed a very high urea concentration of 4.124 mol/l (pH 5.5), and fluid from the proventriculus of two birds had urea concentrations of 382 and 308 mmol/l, respectively. Nine birds were examined during the second episode (Case 2) and, from heparinized heart blood samples collected (n = 5), the mean plasma urea (288 +/- 92.0 mmol/l), ammonia (43.9 +/- 34.2 mmol/l) and uric acid (7.45 +/- 1.99 mmol/l) concentrations were markedly elevated above the reference ranges for all bird species. Proventricular contents (n = 7) similarly contained high concentrations of urea (394 +/- 203 mmol/l) and ammonia (9.3 +/- 15 mmol/l). The probable mechanisms of urea and ammonia toxicity in these birds are discussed.

Rusoff L L; Lank R B; Spillman T E; et al. (1965) **Non-toxicity of urea feeding to horses**. Veterinary medicine, small animal clinician : VM, SAC 60: 1123-1126.

Sarkar SN; Chattopadhyay SK; Majumdar AC. (1995) **Subacute toxicity of urea herbicide, isoproturon, in male rats**. Indian J Exp Biol 33: 851-856. Isoproturon, a substituted phenylurea herbicide, was evaluated for its cumulative toxic effect on growth, organ weight and histomorphology of different organs in adult male rats. The compound (200, 400 and 800 mg/kg/day for 6 days/week), suspended in refined groundnut oil, was administered to rats p.o. for 7 and 10 weeks. There were no definite signs and symptoms of toxicity in treated rats but the herbicide significantly decreased the weekly body weight of rats at 800 mg/kg dose. Isoproturon, in all the three doses, increased the weight of liver in a dose-dependent manner. At 800 mg/kg dose, isoproturon increased the weight of kidney and heart, and decreased the weight of epididymis. Histopathological alterations in the organs were dose-dependent. Salient microscopic changes induced by isoproturon were hepatocellular degenerative changes and focal necrosis in liver, glomerular and tubular degeneration in kidney and haemosiderosis in spleen. Testis showed degeneration and desquamation of cells of germinal layers. Tubular lumens of testis and epididymis exhibited reduced number of spermatids and spermatozoa, respectively, indicating retardation of spermatogenesis.

USSR. (1983) **Effect of urea and creatinine on antitoxic function of the liver in rats**. Krakovskii, ME; Khakimov, ZZ. Deposited Doc., No. VINITI 2632-83. Administration of varying doses of urea (I) [57-13-6] and creatinine (II) [60-27-5] to male rats increased their contents in the blood plasma; the accumulated amts. of I and II corresponded to those seen in acute renal failure. Increasing doses of I and II paralleled with the lengthening of hexenal sleep (a little longer with I). The increase in the hexenal sleep seen with high doses of I and II was associated with decreased activities of major microsomal enzymes responsible for detoxication. 3 Day administration of I decreased amidopyrine N-demethylase [9037-69-8], aniline hydroxylase [9012-80-0], and cytochrome P 450 [9035-51-2] and b5 [9035-39-6] in the liver. I presumably directly affects the cytoplasmic enzymes and cytochromes which ultimately decrease the detoxication function of the liver. Thus, disruption of the antitoxic function of the liver during failure is related to high accumulation of I and II.

Wilczynski M; Owsiejczuk W. (1967) **Investigations of the Toxicity of Urea in Swine**. Medycyna Weterynaryjna 23: 466-467.

Word, JD; Martin, LC; Williams DL; et al. (1969) **Urea toxicity studies in the bovine**. J Anim Sci 29: 786-791.

Zarnke, RL; Taylor, WP, Jr. (1982) **Urea poisoning in free-ranging Alaskan bison**. J Am Vet Med Assoc 181: 1417.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Almeida OD Jr.; Kitay DZ. (1988) **Amniotic fluid urea nitrogen in the prediction of respiratory distress syndrome**. Am J Obstet Gynecol 159: 465-468. Amniotic fluid urea nitrogen levels can be used to predict the neonate not at risk for the development of respiratory distress syndrome. A prospective study of 110 pregnancies compared amniotic fluid urea nitrogen concentrations with the lecithin/sphingomyelin ratio and presence of phosphatidylglycerol. After determination of gestational age, amniotic fluid was analyzed for urea nitrogen concentration and an aliquot was sent to the hospital's commercial laboratory for lecithin/sphingomyelin and phosphatidylglycerol measurement. An amniotic fluid urea nitrogen level less than 7.4 mg/dl predicted the presence of neonatal respiratory distress syndrome in 12 of 13 patients independent of gestational age and birth weight. This assay can be performed at the bedside with simple equipment in 70 seconds at approximately one-fortieth the cost of present standard lung maturity studies.

Anteby SO; Segal S; Polishuk WZ. (1974) **Termination of midtrimester pregnancy by intramniotic injection of urea**. Obstet Gynecol 43: 765-768.

Battaglia F C. (1995) **Amino acid oxidation and urea production rates in fetal life**. Biol Neonate 67: 149-153. Amino acid oxidation and urea production rates are quite different at various stages of development from fetal to adult life. Such information may become increasingly useful in the evaluation of the nutritional state in newborn infants and particularly in very-low-birth-weight infants. A brief commentary on some of the current clinical and basic physiological literature as it relates to amino acid oxidation and urea production is presented. The commentary emphasizes that the literature supports the concept of a higher urea production rate expressed per kilogram body weight in late fetal life as compared with postnatal life. It also discusses some differences for the phases of fetal life, the period of parturition, and the immediate neonatal period.

Beratis NG; Hirschhorn K. (1972) **Properties of placental alkaline phosphatase. 3. Thermostability and urea inhibition of isolated components of the three common phenotypes**. Biochem Genet 6: 1-8.

Binkin NJ; Schulz KF; Grimes DA; et al. (1983) **Urea-prostaglandin versus hypertonic saline for instillation abortion**. Am J Obstet Gynecol 146: 947-952. Authorities have suggested use of a combination of hyperosmolar urea and low-dose prostaglandin F2 alpha as a second-trimester intra-amniotic abortifacient to avoid the disadvantages of hypertonic saline solution. To examine the safety and efficacy of urea-prostaglandin compared with the instillation of saline solution, we analyzed data from a prospective multicenter study conducted in the United States between 1975 and 1978. Both agents were highly effective in producing an abortion. However, urea-prostaglandin had a significantly lower rate of serious complications when compared with saline solution (1.03 versus 2.18 per 100 abortions; p less than 0.001). Urea-prostaglandin also had a significantly shorter induction-to-abortion time (14.2 versus 25.6 hours; p less than 0.001). Urea-prostaglandin, therefore, appears to be superior to hypertonic saline solution as an abortifacient.

Birkett DJ; Conyers RA; Neale FC; et al. (1967) **Action of urea on human alkaline phosphatases: with a description of some automated techniques for the study of enzyme kinetics**. Arch Biochem Biophys 121: 470-479.

Blake DA; Burnett LS; Miyasaki BC; et al. (1976) **Comparative effects of hyperosmolar urea administered by intra-amniotic, intravenous, and intraperitoneal routes in rhesus monkeys**. Am J Obstet Gynecol 124: 239-244. Hypertonic urea solutions (58 per cent weight/volume) were injected

rapidly into ten anesthetized rhesus monkeys at a dose of approximately 2 Gm. per kilogram by the intravenous, intraperitoneal, or intra-amniotic routes. An additional monkey received an intra-amniotic dose of 12.5 Gm. per kilogram. A tracer dose 14C-urea was included for measuring absorption and elimination. The following parameters were monitored before and for four hours after urea injection: arterial and cerebrospinal fluid pressures, heart and respiration rates, urine flow, and urea clearance. Serum electolytes, hematocrit and white count, and cumulative urea excretion were measured for one week. Monkeys were observed for three to six months after injection. At doses up to approximately twice the human dose (on a body weight basis) there were no urea-related deaths and no serious side effects noted in any of the experiments. These results support the suggestion that urea is a relatively safe hyperosmolar agent for inducing midtrimester abortions, especially with regard to inadvertent systemic injection.

Burkman RT; Atienza MF; King TM; et al. (1976) **Intra-amniotic urea and prostaglandin F2 alpha for midtrimester abortion: a modified regimen**. Am J Obstet Gynecol 126: 328-333. A study comparing intra-amniotic urea plus intravenous oxytocin and intra-amniotic urea with 10 mg. prostaglandin F2 alpha was completed. In addition, the results obtained with a further 150 patients receiving urea and prostaglandin are reported. Mean injection-abortion intervals ranged from 15.75 hours for urea-prostaglandin to 18.93 hours for urea-oxytocin. The advantages of urea-prostaglandin and suggested improvements are discussed. Over all, the method appears efficacious though incomplete abortions and cervical laceration are persistent problems.

Burkman RT; Bell WR; Atienza MF; et al. (1977) **Coagulopathy with midtrimester induced abortion: association with hyperosmolar urea administration**. Am J Obstet Gynecol 127: 533-536. Coagulation changes, usually subclinical, have been reported in association with the induction of midtrimester abortion by the administration of intra-amniotic hypertonic sodium chloride, hyperosmolar urea, hyperosmolar urea plus prostaglandin F2alpha, and hyperosmolar ura or hypertonic glucose plus prostaglandin E2. In addition, clinically significant coagulopathy has been described in association with the administration of hypertonic sodium chloride. This study details a three-year experience involving 3,034 cases of midtrimester elective abortion and describes six cases of coagulopathy in association with the administration of hypertonic sodium chloride and two cases in association with the administration of hyperosmolar urea. The significance of these findings and etiologic considerations are discussed.

Chaube, S; Murphy, Ml. (1966) **The effects of hydroxyurea and related compounds on the rat fetus**. Cancer Res 26: 1448-1457.

Conner, EA; Blake, DA; Parmley, TH; et al. (1976) **Efficacy of various locally applied chemicals as contragestational agents in rats**. Contraception 13: 571-582.

Craft I. (1973) **Induction of abortion by combined intra-amniotic urea and prostaglandin E 2 or prostaglandin E 2 alone**. Lancet 1: 1344-1346.

Craft I. (1973) **Intra-amniotic urea and prostaglandin E2 for abortion. A clinical study to determine the efficacy of using a variable prostaglandin dosage**. Prostaglandins 4: 755-763.

Craft I. (1975) **Intra-amniotic urea and low-dose prostaglandin E2 for midtrimester termination**. Lancet 1: 1115-1116. The efficacy and morbidity of injecting a standard dose of urea (80 g.) and a lower dose of prostaglandin E2 (P.G.E2) (2-5 MG) than that used previously have been assesed in 110 patients. All aborted--in a mean time of 10 hours 41 minutes. The average inpatient stay was 3-3 days. 5 patients (4-5%) had a cervical laceration.

Craft I; Bowen-Simpkins P. (1974) **Letter: Adverse reactions to intra-amniotic urea and prostaglandin**. Br Med J 2: 446.

Delecour P; Codoccioui D. (1971) **[Determination of various biochemical components of the amniotic fluid and its relationship to the endangering of the fetus in utero (glucose, urea, pyruvate, creatinine and estriol)]. BESTIMMUNG VERSCHIEDENER BIOCHEMISCHER BESTANDTEILE DES FRUCHTWASSERS UND IHRE BEZIEHUNG ZUR GEFAHRDUNG DES FETEN IN UTERO (GLUKOSE, HARNSTOFF, LAKTAT, PYRUVAT, KREATININ UND OSTRIOL)**. Gynakol Rundsch 11: 216-217.

Deshmukh MA; Prabhoo MR; Sathe AV; et al. (1982) **Coagulation studies following mid-trimester intra-amniotic urea injection**. J Postgrad Med 28: 210-213.

Dollander, A; Marconnet, R; Autelin, R. (1962) **Comparative action of different doses of urea on the development of the chick embryo**. C R Seances Soc Biol Fil 156: 890-892.

Droegemueller W; Chvapil M; Vining J; et al. (1978) **Urea and dilatation of the cervix**. Am J Obstet Gynecol 132: 775-782. The minimal elasticity of the uterine cervix has been related to its high collagen content. By interfering with cohesive forces that hold the collagen fiber bundle together, it is possible to diminish the physical strength of collagen. Urea has been used in collagen chemistry to dissociate collagen by interfering with hydrophobic linkages and hydrogen bonds. This results in a change of mechanical properties such as a decrease in tensile strength and an increase in elasticity. Basic studies of rat tail tendon demonstrated the additive effects of pH, concentration of urea, and temperature on the mechanical characteristics of collagen fibers. In vitro testing was performed using an injection of 30 per cent urea at pH 4. Serial measurements with a spring gauge instrument showed a reduction in cervical resistance. A cervical injection technique has been developed and toxicity of urea studied.

Golditch IM; Solberg N. (1975) **Induction of midtrimester abortion with intraamniotic urea, intravenous oxytocin and laminaria**. J Reprod Med 15: 225-228. Midtrimester abortion was accomplished in 75 patients by the intraamniotic instillation of 80 g of urea and the intravenous administration of oxytocin. In 33 of the patients, laminaria tents were inserted into the cervix. No severe complication occurred; all fetuses were stillborn. A single urea instillation was effective in 94.6% of the patients. The mean instillation-abortion interval was significantly (p less than 0.02) shorter in patients with laminaria tents than in those without. The tents probably prevent cervical rupture in s-me patients. Significant but transient changes occurred in platelet count and blood urea nitrogen one hour after urea instillation. Surgical removal of the placenta was required in 18.7% of the 75 patients; infection occurred in 2.6%. The combined use of urea, oxytocin, and laminaria appears to be an effective and relatively safe method of inducing abortion during the second trimester.

Gombar, VK; Borgstedt, HH; Enslein, K; et al. (1991) **A QSAR model of teratogenesis**. Quantitative Structure Activity Relationships 10: 306-332. Four related QSAR models of teratogenesis in exptl. animals have been developed: one each for heteroarom., carboarom., alicyclic and acyclic compds. The nos. of compds. in these models range from 40 (for the alicyclic model) to 144 (for the carboarom. model). As determined by cross-validation using the leave-one-out, or jackknife, technique, the accuracy of the models in discriminating between teratogens and nonteratogens ranges from 92.4% to 96%. A single overall assessment of exptl. teratogenesis was chosen as the biol. endpoint; taking into account such factors as dosage, maternal toxicity, and affected organ systems remain to be subjects of further studies.

Greenhalfjo; Diggory PL. (1971) **Induction of therapeutic abortion by intra-amniotic injection of urea**. Br Med J 1: 28-29.

Grundy, MF; Craven, ER. (1976) **Consumption coagulopathy after intra-amniotic urea**. Br Med J 2: 677-678.

Guo, L; Zhao, D; Song, Y; et al. (2007) **Reduced urea flux across the blood-testis barrier and early maturation in the male reproductive system in UT-B-null mice**. Am J Physiol Cell Physiol 293: C305-C312. A urea-selective urine-concentrating defect was found in transgenic mice deficient in urea transporter (UT)-B. To determine the role of facilitated urea transport in extrarenal organs expressing UT-B, we studied the kinetics of [(14)C]urea distribution in UT-B-null mice versus wild-type mice. After renal blood flow was disrupted, [(14)C]urea distribution was selectively reduced in testis in UT-B-null mice. Under basal conditions, total testis urea content was 335.4 +/- 43.8 mug in UT-B-null mice versus 196.3 +/- 18.2 mug in wild-type mice ($P < 0.01$). Testis weight in UT-B-null mice (6.6 +/- 0.8 mg/g body wt) was significantly greater than in wild-type mice (4.2 +/- 0.8 mg/g body wt). Elongated spermatids were observed earlier in UT-B-null mice compared with wild type mice on day 24 versus day 32, respectively. First breeding ages in UT-B knockout males (48 +/- 3 days) were also significantly earlier than that in wild-type males (56 +/- 2 days). In competing mating tests with wild-type males and UT-B-null males, all pups carried UT-B-targeted genes, which indicates that all pups were produced from breeding of UT-B-null males. Experiments of the expression of follicle-stimulating hormone receptor (FSHR) and androgen binding protein (ABP) indicated that the development of Sertoli cells was also earlier in UT-B-null mice than that in wild-type mice. These results suggest that UT-B plays an important role in eliminating urea produced by Sertoli cells and that UT-B deletion causes both urea accumulation in the testis and early maturation of the male reproductive system. The UT-B knockout mouse may be a useful experimental model to define the molecular mechanisms of early puberty.

Kafrissen ME; Schulz KF; Grimes DA; et al. (1984) **Midtrimester abortion. Intra-amniotic instillation of hyperosmolar urea and prostaglandin F2 alpha v dilatation and evacuation**. JAMA 251: 916-919. Although dilatation and evacuation (D&E) is currently the most common method of midtrimester abortion in the United States, the intra-amniotic instillation of hyperosmolar urea and prostaglandin F2 alpha combined (U-P) has been proposed as a safer technique. To evaluate the comparative safety of U-P and D&E, we analyzed 2,805 U-P and 9,572 D&E abortions at 13 to 24 menstrual weeks' gestation. The U-P procedure resulted in significantly more serious complications than D&E (1.03 v 0.49 per 100 abortions). After adjusting for patient age, race, parity, follow-up information, and preexisting conditions, the relative risk of serious complications associated with U-P was 1.9 (95% confidence interval, 1.2 to 3.1). This advantage for D&E stems from its applicability to the 13- to 16-week interval. Although D&E appears to be safer overall in the midtrimester, for women obtaining abortion after 16 weeks, the rates of serious complications were comparable, with a relative risk of 1.0 (95% confidence interval, 0.4 to 2.5).

Kaneda, M; Aoyama, H; Suzuki, R; et al. (1980) **Teratogenicity of ureas in rats and mice**. Teratology 22: 13A.

King TM; Atienza MF; Burkman RT; et al. (1974) **The synergistic activity of intra-amniotic prostaglandin F2 alpha and urea in the midtrimester elective abortion**. Am J Obstet Gynecol 120: 704-718.

King TM; Dubin NH; Atienza MF; et al. (1977) **Intra-amniotic urea and prostaglandin F2alpha for midtrimester abortion: clinical and laboratory evaluation**. Am J Obstet Gynecol 129: 817-824. The

clinical management of the elective midtrimester abortion continues to be unsatisfactory as judged by either national mortality or morbidity rates. This report documents the results of a randomized series of 19 midtrimester abortions induced by either intra-amniotic hyperosmolar urea and 5 mg. of prostaglandin F2alpha (PGF2alpha) or intra-amniotic hyperosmolar urea alone. Pertinent clinical characteristics and biochemical determinations were compared between these two groups. A series of 150 patients were then treated with urea and 5 mg. of PGF2alpha. The clinical results of this series of patients are presented and compared with a previous group who had urea and 10 mg. of PGF2alpha. These studies demonstrate that 5 mg. of PGF2alpha with 80 Gm. of urea achieves injection-abortion intervals that are less than 24 hours.

Kovasznay BM; Burkman RT; Atienza MF; et al. (1979) **Intravascular spill of hyperosmolar urea during induced midtrimester abortion**. Obstet Gynecol 53: 127-130. Intravascular spill of hypertonic sodium chloride and the resultant serious and occasionally fatal consquences have been reported in association with induced midtrimester abortion. This report details 3 cases of intravascular spill of hyperosmolar urea. Although urea may pose less concern, because of its ability to readily cross cell membranes and its action as an osmotic diuretic, inadvertent intravascular spill can produce symptoms that include nausea, headache, sensations of warmth, and intense uterine cramping. In addition, abnormal blood pigments may occasionally be noted in the urine. Treatment includes intravenous hydration, careful monitoring of fluid/electrolyte balance and renal function, and avoiding the use of oxytocic agents.

McKay E; Kilpatrick S J. (1964) **Maternal and Infant Plasma Urea at Delivery**. The Journal of obstetrics and gynaecology of the British Commonwealth 71: 449-452.

Ordonez, A; Parkinson, TJ; Matthew, C; et al. (2007) **Effects of application in spring of urea fertiliser on aspects of reproductive performance of pasture-fed dairy cows**. N Z Vet J 55: 69-76. AIMS: To assess if raising concentrations of crude protein (CP) in pasture in spring by the frequent application of urea fertiliser would affect ovarian follicular dynamics, luteal function, onset of oestrus and reproductive performance of dairy cows under farming conditions in New Zealand. METHODS: Spring-calved dairy cows were grazed for 101 days in paddocks that were either not fertilised (Control; n=20) during the course of the study, or were fertilised with 40-50 kg nitrogen (N)/ha every 4-6 weeks (High-N; n=20). Similar generous pasture allowances were offered to both groups. Concentrations of CP in pasture, urea in serum and progesterone in milk were measured. Ovarian follicular and luteal dynamics were determined using ultrasonography. Oestrous behaviour and the number, time and outcome of inseminations were also recorded. RESULTS: Mean concentrations of CP in pasture and urea in serum was higher in the High-N than the Control group (25.2 vs 21.6 and 8.3 vs 5.4 mmol/L for CP and urea, respectively; $p<0.001$). Intervals between calving and first oestrus, first insemination and conception, the time of first emergence of a dominant follicle, milk progesterone concentration, and the diameter of the corpus luteum (CL) in the first luteal phase did not differ significantly between groups. The interval from calving to first ovulation tended ($p=0.10$) to be lower and the diameter of the dominant follicle of the oestrous cycle at which cows conceived was greater ($p=0.02$) in Control than High-N cows. CONCLUSIONS: The use of large amounts of urea fertiliser during spring and the consequent increases in concentrations of CP in pasture and urea in serum did not negatively affect any of the parameters of reproductive performance of pasture-fed dairy cows that were assessed in this study.

Pushkina IK; Bereznyak IV; Tsirkova NL; et al. (1982) **Effect of working conditions and family life on the health status and reproductive function of women in the production and use of urea resins**. Vopr Gig Tr Nov Tekhnol Protsessami Mashinostroit Khim Prom-sti, [Mater Mosk Nauchn Konf Osnovn Probl Gig Tr] 35: 87-91. In the manufacture and application of urea resins, female workers are exposed to high concns. of HCHO [50-00-0], MeOH [67-56-1] vapors, NH3, heat, noise, and functional

stress, which, in combination with fatigue and emotional stress caused by poor living conditions, result in severe disorders of the autonomic nervous system and digestive tract, as well as in the development of cholestasis and liver dysfunction. There was a significant increase in the incidence of toxicoses and bleeding during labor in pregnant workers, as compared to a control group. Also, the offspring of exposed females had a significantly decreased weight and were predominantly males.

Rhoads, ML; Rhoads, RP; Gilber, RO; et al. (2006) **Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows**. Anim Reprod Sci 91: 1-10. High plasma urea nitrogen (PUN) concentrations are associated with decreased fertility in lactating dairy cows. Our objective was to evaluate the quality of embryos flushed from superovulated lactating cows having moderate or high PUN concentrations. Subsequent embryo survival was determined after transfer to recipient heifers with either low or high PUN. Lactating Holstein dairy cows (n = 23; 50-120 days in milk) were randomly assigned to one of two diets designed to result in moderate or high PUN concentrations (15.5 +/- 0.7 and 24.4 +/- 1.0 mg/dl, respectively; P < 0.001) and were fed for 30 days before embryo flushing and recovery. Embryos (n = 94) were evaluated morphologically, frozen and subsequently transferred into synchronized virgin heifers that were fed one of two diets designed to result in either low or high PUN concentrations (7.7 +/- 0.9 and 25.2 +/- 1.5 mg/dl, respectively; P < 0.001; 2 x 2 factorial design). The number, quality and stage of development of recovered embryos were similar for cows with moderate or high PUN. Transfer of embryos from moderate PUN donor cows resulted in a higher pregnancy rate (35%; P < 0.02) than the transfer of embryos from high PUN donor cows (11%). Pregnancy rate was not affected by either recipient diet or the interaction of donor and recipient diets (P > 0.05). These results indicate that high PUN concentrations in lactating dairy cows decrease embryo viability through effects exerted on the oocyte or embryo before recovery from the uterus 7 days after insemination.

Ross AH; Whitehouse WL. (1974) **Letter: Adverse reactions to intra-amniotic urea and prostaglandin**. Br Med J 1: 642.

Seipelt, H; Zoellner, K; Hilgenfeld, E; et al. (1969) **[Studies on kidneys of newborn rats after chronic urea administration to the mother]. UNTERSUCHUNGEN AN NIEREN NEUGEBORENER RATTEN NACH CHRONISCHER HARNSTOFFAPPLIKATION BEIM MUTTERTIER**. Z Urol Nephrol 62: 623-627.

Shor V; Gitler M; Karchmer S. (1972) **[Study of feto-maternal metabolic relations. IV. Urea, uric acid and creatinine in the amniotic fluid of normal pregnancy]. ESTUDIO DE LAS RELACIONES METABOLICAS FETO MATERNAS. IV. UREA ACIDO URICO Y CREATININA EN EL LIQUIDO AMNIOTICO DEL EMBARAZO NORMAL**. Ginecol Obstet Mex 31: 467-474.

Smith RJ; Newton J. (1973) **A double-blind study of intra-amniotic urea and hypertonic saline for therapeutic abortion**. J Obstet Gynaecol Br Commonw 80: 135-137.

Teramoto, S; Kaneda, M; Aoyama, H; et al. (1981) **Correlation between the molecular structure of N-alkylureas and N-alkylthioureas and their teratogenic properties**. Teratology 23: 335-342. Eleven urea compounds were administered individually as a single oral dose to rats either on day 12 or 14 and to mice on day 10 of pregnancy. 1-Methylurea and 1-ethylurea were not teratogenic in either of the animal species. Administration of 1-methylthiourea and 1-ethylthiourea, however, resulted in high incidences of malformations in the rat but not in the mouse fetuses. The types of malformation were similar to those produced by the known teratogen ethylenethiourea. Methylated ureas such as 1,3-dimethylurea, 1,3-dimethylthiourea, 1,1,3,3-tetramethylurea, and 1,1,3,3-tetramethylthiourea were fetotoxic and produced

malformations in the tail, palate, or extremities of the surviving rat and/or mouse fetuses. These findings suggest that the teratogenic properties of N-alkylureas can be categorized into two groups, i.e., mono-alkylated thioureas and methylated ureas or thioureas.

Weinberg PC; Linman JE; Linman SK. (1975) **Intraamniotic urea for induction of midtrimester pregnancy termination: a further evaluation**. Obstet Gynecol 45: 320-324. Forty percent hyperosmolar urea solution was used intraamniotically to induce midtrimester pregnancy termination in 508 patients. The mean injection-abortion interval was 43.4 hours in those patients aborting within 7 days (85.8% of the total group); 76% of the group aborted within 72 hours. Complications from the procedure included endometritis, hemorrhage, and nausea and vomiting; 29.3% of the patients required operative completion of the abortion (placental removal 12 hours after passage of the fetus). There were no cases of hypernatremia, cardiac arrest or collapse, clinically evident coagulopathies, nor cervical lacerations. This study supports the conclusion that urea is a safer intraamniotic solution than hypertonic saline for midtrimester pregnancy termination.

Wilson WB Jr. (1978) **Midtrimester abortion with urea, prostaglandin F2alpha, laminaria, and oxytocin. A new regimen**. Obstet Gynecol 51: 699-701. This study was undertaken to determine a method of amino infusion that would 1) produce abortion within 12 hours; 2) be relatively free from risks of coagulapathy and electrolyte imbalance; 3) not result in delivery of liveborns; and 4) incur minimal gastrointestinal side effects from prostaglandin. Patients were randomly assigned to 1 of 3 groups unless history and examination revealed a contraindication to the use of prostaglandin. Three infusions were used: prostaglandin alone, urea alone, and a combination of urea and prostaglandin. All patients had pre-infusion laminaria inserted and all received oxytocin following infusion. There was a significant difference in instillation to abortion time when comparing the three groups and a marked reduction in gastrointestinal side effects using a lower dosage of prostaglandin. The synergistic effect of urea and prostaglandin F2alpha, previously demonstrated was further enhanced by the use of oxytocin and laminaria. This produced a mean instillation to abortion time significantly shorter than previous studies have shown and, indeed, offers a means of second trimester abortion suitable for use in ambulatory surgery facilities, precluding the high cost of inpatient care.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

4.4.1 Genotoxicity

Kommadath, A; Sharma, A; Jakhar, KK. (2001) **Hepatotoxic, nephrotoxic and genotoxic effects in mice fed urea adulterated milk**. Indian J Dairy Sci 54: 316-321. The genotoxic effects of urea at levels detected in adulterated milk were investigated by in vivo chromosomal aberration test using mouse bone marrow assay. Forty-eight male mice (Swiss Albino) weighing 15-35 g were divided into 4 groups of twelve animals each. Mice of groups I, II and III were fed 0.1 mL milk adulterated with 0.73 mg, 0.365 mg and 0.1825 mg urea, resp. for 28 days by esophageal intubation. These levels were calculated to simulate the level of urea adulterated milk consumption by humans. Group IV (control) mice were fed 0.1 mL natural cow milk for the same period. Three animals were sacrificed from each group on 7th, 14th, 21st and 28th day of experiment and metaphase spreads prepared for evaluation of mitotic index and chromosomal aberrations. Liver and kidney samples of mice were collected on 7th and 28th day of experiment for histopathol. study. Among the chromosomal aberrations observed, centromeric breaks and polyploidy were most common. The differences in mitotic indexes and frequencies of chromosomal aberrations between the 4 groups at different intervals were found to be statistically nonsignificant.

Histopathol. changes were observed in the liver and kidney of all treated groups with severity of lesions increasing with duration of feeding. It is concluded from the present study conducted in mice that urea consumption of adulterated milk for a short duration of even 7 days can produce hepatotoxicity and nephrotoxicity, but genotoxicity was not observed even after 28 days of the experiment.

Kultz, D; Chakravarty, D. (2001) **Hyperosmolality in the form of elevated NaCl but not urea causes DNA damage in murine kidney cells**. Proc Natl Acad Sci USA 98: 1999-2004. This study demonstrates, by using neutral comet assay and pulsed field gel electrophoresis, that hyperosmotic stress causes DNA damage in the form of double strand breaks (dsb). Different solutes increase the rate of DNA dsb to different degrees at identical strengths of hyperosmolality. Hyperosmolality in the form of elevated NaCl (HNa) is most potent in this regard, whereas hyperosmolality in the form of elevated urea (HU) does not cause DNA dsb. The amount of DNA dsb increases significantly as early as 15 min after the onset of HNa. By using neutral comet and DNA ladder assays, we show that this rapid induction of DNA damage is not attributable to apoptosis. We demonstrate that renal inner medullary cells are able to efficiently repair hyperosmotic DNA damage within 48 h after exposure to hyperosmolality. DNA repair correlates with cell survival and is repressed by 25 microM LY294002, an inhibitor of DNA-activated protein kinases. These results strongly suggest that the hyperosmotic stress resistance of renal inner medullary cells is based not only on adaptations that protect cellular proteins from osmotic damage but, in addition, on adaptations that compensate DNA damage and maintain genomic integrity.

Lintner C; Roman C. (2006) **Testing of certain high production volume chemicals**. Fed Regist 71: 13708-13735. USEPA promulgated a final rule under the Toxic Substances Control Act (TSCA) which requires manufacturers, importers, and processors of 17 high production volume (HPV) chems. to conduct acute toxicity, repeat-dose toxicity, developmental and reproductive toxicity, genetic toxicity (gene mutations and chromosomal aberrations), ecotoxicity (fish, Daphnia, algae), and environmental fate (including 5 tests for physicochem. properties and biodegrdn.) testing. EPA determined each of the 17 chems. included in this final rule is produced in substantial quantities and there is or may be substantial human exposure to each of them. EPA determined there are insufficient data to reasonably determine or predict health or environmental effects from the manufacture, distribution in commerce, processing, use, or disposal of these chems., or any combination of these activities. EPA concluded this testing program is necessary and appropriate for developing such data. Data developed under this final rule will provide critical information about the environmental fate and potential hazards of these chems., which, when combined with exposure and use information, will allow EPA and others to evaluate potential health and environmental risks and take appropriate actions. Persons who export or intend to export any chemical included in this final rule, regardless of the form in which it is exported, are subject to export notification requirements of TSCA Section 12(b).

Maddock, AL; Westenfelder, C. (1996) **Urea induces the heat shock response in human neuroblastoma cells**. J Am Soc Nephrol 7: 275-282. Uremic encephalopathy is a complication of renal failure that reflects stresses exerted by as yet poorly defined uremic toxins. All cells respond to stresses by undergoing the "heat shock" response. Although urea kinetics and creatinine concentration are routinely used to assess dialysis adequacy, the roles of urea and creatinine as uremic toxins remain controversial. To investigate their potential roles in uremic encephalopathy, cultured human neuroblastoma cells (SK-N-SH) were exposed to 0.5 to 14 mg/dL creatinine, or to 20 to 200 mg/dL urea, or to mannitol, NaCl, or glycerol at equivalent osmolalities for 30 min to 48 h, and the induction of Hsp72 (heat shock) protein was used as a marker of cell stress. Although creatinine failed to elicit a heat shock response, urea in clinically relevant concentrations (40 to 200 mg/dL) induced it at 30 min. The response peaked at 10 h and returned to zero by 48 h. Cells exposed to equivalent osmolalities of mannitol, NaCl, or glycerol failed to exhibit this response. Protein extracts from cells exposed to urea

showed significant carbamylation that increased as a function of time. These results demonstrate: (1) that urea is neurotoxic in vitro and that creatinine is not: (2) that the insult urea causes is not simply the result of hypertonicity; but rather (3) that urea, via breakdown to cyanate and ammonium ions, may cause cell stress because of its ability to cause carbamylation of cellular proteins. The cells attenuation of the heat shock response after 10 h of exposure to urea suggests that they can adapt to the presence of urea or carbamylation. This may explain, in part, why the same degree of azotemia causes fewer neurological symptoms in patients with chronic as opposed to acute renal failure.

Moriya, M; Ohta, T; Watanabe, K; et al. (1983) **Further mutagenicity studies on pesticides in bacterial reversion assay systems**. Mutation Research 116: 185-216. A total of 228 pesticides (88 insecticides, 60 fungicides, 62 herbicides, 12 plant-growth regulators, 3 metabolites, and 3 other compds.) were tested for mutagenicity in bacterial reversion-assay systems with 5 strains (TA 100, 98, 1535, 1537, and 1538) of Salmonella typhimurium and a strain (WP2 hcr) of Escherichia coli. Fifty pesticides (25 insecticides, 20 fungicides, 3 herbicides, 1 plant-growth regulator, and 1 other compound) were mutagenic, 5 of which required metabolic activation (S9 mix). Among various chemical groups, organic phosphates, halogenated alkanes, and dithiocarbamates had higher ratios of mutagens. Although 22 of the pesticides tested have been reported to be carcinogenic, 7 of them, i.e., captan [133-06-2], DBCP [96-12-8], EDB [106-93-4], EDC [107-06-2], ETU [96-45-7], HEH [109-84-2], and nitrofen [1836-75-5], were detected as mutagens. Most of the 15 nonmutagenic carcinogens were organochlorine pesticides such as ?-BHC [319-84-6], chlorobenzilate [510-15-6], DDT [50-29-3], dieldrin [60-57-1] and quintozene [82-68-8].

Okada, M; Kobayashi, Y. (1989) **Fine structural alterations in mouse pituitary intermediate lobe cells following oral urea administration**. Zoolog Sci 6: 4-6.

Topham, JC. (1980) **Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens?** Mutat Res 74: 379-387. The results of testing 54 compounds including 19 carcinogen/non-carcinogen pairs from a wide range of chemical classes are reported. Many carcinogens did not induce increases in abnormal sperm heads. In contrast compounds known to induce transmissible genetic damage in whole animals invariably induced dose-dependent large increases in the incidence of abnormal sperm heads. The test may be useful in assisting discrimination between compounds that only cause mutations in isolated cell systems from those which constitute a real genetic hazard for whole mammals.

Umeda, M; Noda, K; Ono, T. (1980) **Inhibition of metabolic cooperation in Chinese hamster cells by various chemical sincluding tumor promoters**. Jpn J Cancer Res 71: 614-620.

4.4.2 Cardiovascular

Abaurre, PF; Stefanon, I; Mill, JG; et al. (1992) **Electromechanical effects of urea on the isolated rat heart**. Brazilian Journal of Medical and Biological Research 25: 717-726. Langendorff-perfused isolated hearts and left ventricle papillary muscles from 3-mo-old albino rats of both sexes were studied before and after a 30-min treatment with 17 mM urea added to the medium, a concentration equivalent to a plasma level of 100 mg/dL. To determine whether the effects observed after the addition of urea were produced by an increase in tonicity, the study was repeated using 17 mM sucrose. Mech. studies on the papillary muscles showed that isometric force development and its first time derivative decreased after washing out urea from the bath (F = 9.73 g/mm2 to 7.47 and dF/dt =

66.8 to 56.7 g (mm2)s-1, resp. Inotropic responses to isoproterenol and increased extracellular calcium after urea treatment reached values similar to those obtained before urea treatment. Thus, the effect of isoproterenol and calcium was stronger than that obtained before urea treatment. In Langendorff-perfused hearts, the spontaneous heart rate did not change after urea or sucrose treatment. Urea promoted a decrease in the left ventricle isovolumic systolic pressure (39.7 to 26.1 mmHg) and a reduction of total QRS amplitude. In both papillary muscles and isovolumic perfused hearts, contractile responses resulting from changes in extracellular sodium concentration were reduced after urea treatment. The increased osmolarity due to sucrose did not produce any changes n electromech. activities. Although 17 mM, which reduces isometric force and isovolumic pressure development and modifies the ECG, is well below the concentration required to modify protein conformation in vitro, the present results suggest that its action could be explained by an effect at the sarcolemmal level.

Cirillo M; Lombardi C; Laurenzi M; et al. (2002) **Relation of urinary urea to blood pressure: interaction with urinary sodium**. J Hum Hypertens 16: 205-212. A previous study reported that urinary markers of protein intake are inversely related to blood pressure via unknown mechanisms. In man and rats, protein intake affects renal function and increases renal sodium excretion. The present study investigates the relation between markers of protein intake and blood pressure and the possible role of sodium in this relation. Blood pressure status, overnight urinary urea as index of protein intake, urinary and plasma sodium, and other variables were measured in a population sample of 3705 men and women, aged 25-74 years, without high plasma creatinine. Urinary urea was inversely related to blood pressure and hypertension: in multivariate analyses, 6.5 mmol/h higher urinary urea (about one s.d. in men and women) was related to 4.25 mm Hg lower systolic blood pressure (95% confidence interval = 1.34-8.49), and to 0.65 lower risk of hypertension (95% CI 0.34-0.87). An interaction was found between overnight urinary sodium and the relation of urinary urea to blood pressure: the relation was significant only in persons with overnight urinary sodium above the median. Urinary urea was significantly and inversely also related to plasma sodium. Data confirm an inverse relation to blood pressure of protein intake as measured by urinary urea. The possibility of sodium-related mechanisms is supported by the interaction of urinary sodium with the relation and by the inverse association of urinary urea with plasma sodium. The hypothesis is made that high protein intake could counteract sodium-dependent blood pressure rise via stimulation of renal sodium excretion.

Cuparencu B; Grosu L; Birsan E T. (1961) **Research on the pharmacodynamic effects of urea on organs with smooth musculature**. Acta biologica et medica Germanica 6: 117-122.

Cuparencu B; Grosu L; Tomus L; et al. (1961) **Research on the pharmacodynamic effects of urea on the cardiovascular system**. Acta biologica et medica Germanica 6: 123-140.

Davis JW; McField JR; Phillips PE; et al. (1972) **Guanidinosuccinic acid on human platelet. Effects of exogenous urea, creatinine, and aggregation in vitro**. Blood 39: 388-397.

Esaian, AM; Titova, VA; Shishkina, LI; et al. (1997) **Effect of urea on the course of experimental uremia in subtotally nephrectomized rats**. Patol Fiziol Eksp Ter 2: 39-41. Increased plasma levels of urea appear to have an important role in the progression of renal failure, as shown in Wistar rats with exptl. uremia induced by subtotal nephrectomy. Renal structural lesions and dysfunction were the same as in rats fed a high-protein diet and in those on a low-protein diet supplemented with urea. The changes were much less in the control group of rats fed a low-protein diet alone. The findings suggest that high blood urea concns. appear to be a cause of intraglomerular hemodynamic disturbances in renal failure.

Kaji DM; Lim J; Shilkoff W; et al. (1998) **Urea inhibits the Na-K pump in human erythrocytes**. J Membr Biol 165: 125-131. Recent studies have established that urea alters the activity of several volume-sensitive cation transport pathways. However, it has remained unclear whether urea has any effect on transport pathways that are not volume-sensitive. The authors examined the effect of urea on Na-K pump in the human erythrocytes. In cells from nine subjects, 500 mM urea inhibited 52 (10% of the pump activity measured as the ouabain-sensitive (OS) K influx. Urea inhibited the OS K influx reversibly, in a concentration-dependent manner. [3H] ouabain binding, a measure of the number of Na-K pump sites remained unchanged with urea. Urea decreased the Vmax for ouabain-sensitive K influx, but did not alter the apparent Km for external K. Furthermore, urea did not alter the apparent Km for intracellular Na. The ion turnover per pump site was decreased in the presence of urea. Thus, physiol. relevant urea concentration inhibit the Na-K pump in human erythrocyte. The inhibition of the Na-K pump by urea suggests that the effects of urea may not be limited to volume-sensitive transporters, but may be more widespread.

Massy, ZA; Ivanovski, O; Nguyen-Khoa, T; et al. (2005) **Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice**. J Am Soc Nephrol 16: 109-116. Chronic renal failure (CRF) favors the development of atherosclerosis and excessive calcification of atheromatous lesions. CRF was induced in apolipoprotein E knockout (apoE(-/-)) mice to study (1) a possible acceleration of aortic atherosclerosis, (2) the degree and type of vascular calcification, and (3) factors involved in the calcification process. For creating CRF, 8-wk-old apolipoprotein E gene knockout (apoE(-/-)) mice underwent partial kidney ablation. Control animals underwent sham operation. Aortic atherosclerotic plaques and calcification were evaluated using quantitative morphologic image processing. At 6 wk after nephrectomy, CRF mice had significantly higher serum urea, cholesterol, and triglyceride concentrations than non-CRF controls. The serum levels of advanced oxidation protein products were elevated in the uremic group and were correlated with serum urea levels. Atherosclerotic lesions in thoracic aorta were significantly larger in uremic apoE(-/-) mice than in nonuremic controls. The relative proportion of calcified area to total surface area of both atherosclerotic lesions and lesion-free vascular tissue was increased in aortic root of uremic apoE(-/-) mice when compared with controls. The calcium deposits were made of hydroxyapatite and calcite crystals. In addition, plaques from uremic animals showed a significant increase in collagen content, whereas the degree of macrophage infiltration was comparable in both groups. There was no difference in mean arterial BP. These findings demonstrate that CRF aggravates atherosclerosis in apoE(-/-) mice. Moreover, CRF enhances arterial calcification at both atheromatous intimal sites and atheroma-free medial sites. We anticipate that this experimental model will be useful to test treatment strategies aimed at decreasing the accelerated atherosclerosis and arterial calcification in uremia.

Matteo RS; Ravin MB. (1966) **The osmotic fragility of human red cells caused by urea administration during hypothermia**. Anesthesiology 27: 318-320.

Moeslinger, T; Spieckermann, PG. (2001) **Urea-induced inducible nitric oxide synthase inhibition and macrophage proliferation**. Kidney Int Suppl 78: S2-8. BACKGROUND: Atherosclerosis is a major cause of morbidity and mortality in chronic renal failure and is associated with the proliferation of macrophages within atherosclerotic lesions. METHODS: Because the progression of atherosclerosis as a consequence of decreased nitric oxide (NO) synthesis has been described, we investigated the correlation between the inhibition of inducible NO synthase (iNOS) by urea, macrophage proliferation as assayed by cell counting, tritiated-thymidine incorporation and measurement of cell protein, and macrophage apoptosis. RESULTS: Urea induces a dose-dependent inhibition of inducible NO synthesis in lipopolysaccharide-stimulated mouse macrophages (RAW 264.7) with concomitant macrophage proliferation. Macrophage proliferation as determined by cell counting became statistically significant at

60 mmol/L urea corresponding to a blood urea nitrogen level of 180 mg/100 mL, concentrations seen in uremic patients. iNOS protein expression showed a dose-dependent reduction, as revealed by immunoblotting when cells were incubated with increasing amounts of urea. The decrease of cytosolic DNA fragments in stimulated macrophages incubated with urea shows that the proliferative actions of urea are associated with a decrease of diminished NO-mediated apoptosis. CONCLUSIONS: These data demonstrate that inhibition of iNOS-dependent NO production caused by urea enhances macrophage proliferation as a consequence of diminished NO-mediated apoptosis. This fact may be important for the development of atherosclerotic lesions during chronic renal failure and is in accordance with recently published studies showing that under conditions with decreased constitutive NOS activity, iNOS might substitute the synthesis of NO. iNOS expression in vascular smooth muscle cells and macrophages is supposed to prevent restenosis following angioplasty or heart transplant vasculopathy. This is supported by the fact that specific inhibition of endogenous iNOS activity with L-N6-(1-iminoethly)-lysine accelerates the progression of vasculopathy in transplantation atherosclerosis.

Ok, E; Basnakian, AG; Apostolov, EO; et al. (2005) **Carbamylated low-density lipoprotein induces death of endothelial cells: a link to atherosclerosis in patients with kidney disease**. Kidney Int 68: 173-178. The presence of accelerated atherosclerosis in patients with kidney disease cannot be entirely explained by traditional cardiovascular risk factors. Exposure to urea, which is normally present in human blood plasma and elevated in patients with kidney disease, leads to the carbamylation of proteins. We postulated that low-d. lipoprotein (LDL) carbamylated by urea has biol. effects relevant to atherosclerosis. To produce carbamylated LDL (cLDL), human native LDL (nLDL) was chemical modified in vitro by exposure to potassium cyanate. Human coronary artery endothelial cells (HCAECs) and human coronary artery smooth muscle cells (CASMCs) were treated in vitro with cLDL or nLDL. Irreversible cell death was measured using the lactate dehydrogenase (LDH) assay, apoptosis was assessed by annexin V binding, and proliferation was determined using bromodeoxyuridine (BrdU) incorporation. Total plasma protein carbamylation and plasma cLDL were measured in hemodialysis patients using the homocitrulline assay and ELISA. Our studies demonstrated that cLDL but not nLDL induced dose-dependent vascular cell injuries relevant to atherosclerosis, which included the proliferation of vascular smooth muscle cells and endothelial cell death. Under light microscopy, endothelial cells treated with cLDL showed signs of morphol. alterations. The injury to endothelial cells measured by LDH release was time-dependent and correlated with the degree of LDL carbamylation. At least a part of the endothelial cell population treated with cLDL died by apoptosis. In patients with advanced renal disease on hemodialysis, total plasma protein carbamylation and plasma cLDL were several times higher than in control healthy individuals. Collectively these data suggest the potential role of carbamylated LDL in accelerated atherosclerosis in patients with chronic renal disease and, possibly, in healthy individuals.

Sahach, VF; Kotsiuruba, AV; Baziliuk, OV; et al. (2004) **[Inhibitors of arginase pathway in L-arginine metabolism as a new class of antihypertensive drugs: action of urea on oxidative and nonoxidative metabolism of L-arginine and vascular tone in chronic hypertension]**. Fiziol Zh 50: 9-18. It has been studied an action of chronic urea introduction (40 mg/kg, 14 and 28 days) as arginase inhibitor on nonoxidative (arginase activity, urea, polyamines content) and oxidative (NOS activity, nitrite- and nitrate-anion content) metabolism of L-arginine in aorta, heart, plasma and erythrocytes of SHR. It has been shown that urea is an inhibitor of not arginase only, but also ornitinedecarboxilase (ODK) reactions, limiting L-arginine consumption for urea and polyamines synthesis and thus facilitating its utilization for nitric oxide synthesis by NOS. These exogenous effects of urea are not accompanied by amelioration of tissue ischaemization within cardiovascular system. It has been shown that exogenous urea down regulate blood pressure without any normalization of endothelim-dependent reactions of smooth muscle cells on acetylcholine in SHR.

Wang H; Ikeda K; Kihara M; et al. (1984) **Effect of dietary urea on blood pressure in spontaneously hypertensive rats**. Clin Exp Pharmacol Physiol 11: 555-561. The feeding of a normal diet containing 13.5% urea (in place of protein in a high protein diet) attenuated the development of severe hypertension and decreased the incidence of stroke in spontaneously hypertensive rats (SHR), when 1% NaCl solution was given to them. The urea not only increased urine volume, but also increased urinary sodium excretion in SHR given 1% NaCl for drinking. Although there was no obvious difference in erythrocyte size between the urea and the control groups, there was a significant inverse correlation between plasma urea level and erythrocyte size. These results suggest that a high protein diet reduced blood pressure partly through the diuretic effect of urea, the common metabolite of various proteins.

4.4.3 Other

Andrade CV; Mello MA; Duarte MEL. (2001) **High concentrations of urea alters the normal patterns of chondrogenesis of limb mesenchyme in vitro**. J Bone Miner Res, 16: 12-16.

Aydemir T. (2003) **The inhibition effects of some chemicals and pesticides on human erythrocyte catalase activity in vitro**. Energy Educ Sci Technol 12: 103-110. Catalase plays a significant role in detoxication of hydrogen peroxide in several metabolic reactions. In the present study catalase was extracted and purified 175 fold from human erythrocytes by (NH4)2SO4 precipitation, ethanol-chloroform treatment and successive chromatogs. on CM-cellulose, Sephadex G-200. The purification of enzyme was controlled with SDS polyacrylamide gel electrophoresis. The effect of some herbicides and some chems. has been investigated on human erythrocyte catalase enzyme. I50 values of chems. caused inhibition were determined by means of activity percentage (I) diagrams. This activity of purified catalase is strongly inhibited by azide, cyanide, ?-mercaptoethanol. Pesticides having the strongest inhibitory effects on human erythrocyte catalase were observed to be Hektavin and Polyram. Fifty percent inhibition was found to be 1.25.10-3 and 1.00.10-3 M these pesticides. Agrosban, Gunner, Mospilan, Shavit, Versal and Ekatin weakly inhibited catalase enzyme.

Barbolini G; Bisetti A; Lombardi R; et al. (1964) **[Experimental Uremic Pneumopathy in the Rabbit. Morphologic and Dynamic Study with C 14-Urea]. PNEUMOPATIA UREMICA SPERIMENTALE NEL CONIGLIO. STUDIO MORFOLOGICO E DINAMICO CON UREA-14 C**. Arch De Vecchi Anat Patol 44: 157-175.

Bauer F. (1970) **The use of urea in exudative otitis media**. Otolaryngol Clin North Am 3: 67-78.

Bauer F. (1975) **Tubal function in the glue ear: urea for glue ears**. J Laryngol Otol 89: 63-71.

Binet L; Dejours P. (1953) **[Morphologic development of the kidney and noxiousness of urea in the rat]. DEVELOPPEMENT MORPHOLOGIQUE DU REIN ET NOCIVITE DE L'UREE CHEZ LE RAT**. C R Hebd Seances Acad Sci 236: 1829-1832.

Bozhko GKh; Voloshin PV; Galushko EI. (1983) **[Study of serum protein aggregation after treatment with urea]. ISSLEDOVANIE AGREGATSII BELKOV SYVOROTKI KROVI POD VLIIANIEM MOCHEVINY**. Ukr Biokhim Zh 55: 325-328. Gel chromatography and electrophoresis in polyacrylamide gel show that urea in vitro induces aggregation of blood proteins, with their content unchanged. The aggregation does not depend on the fibrinogen available. Comparison of the data on the effect of urea, DS-Na and thermal denaturation on the protein aggregation suggests that the aggregation is induced by local conformation transformations in the structure of certain protein molecules. At the same time, the data obtained do not permit neglecting the role of denaturation in the aggregation.

Chandra M; Singh B; Singh N; et al. (1984) **Hematological changes in nephritis in poultry induced by diets high in protein, high in calcium, containing urea, or deficient in vitamin A**. Poult Sci 63: 710-716. Nephritis was induced in 300, 18-day-old male Arbor Acre broiler chicks by feeding diets high (42.28%) in protein, high (3.27%) in calcium, containing urea (5%), or deficient in vitamin A. Various hematological parameters were studied at weekly intervals. Normocytic-normochromic anemia, characterized by a decrease in total erythrocyte counts, hemoglobin, packed cell volume, and an increase in erythrocyte sedimentation rate, was evident in the birds kept on diets high in protein, high in calcium, or deficient in vitamin A. Increased total erythrocytes, hemoglobin packed cell volume, and erythrocyte sedimentation rate was observed in birds fed urea. Differential leucocyte counts revealed lymphopenia, heterophilia and monocytosis in birds kept on diets high in protein, containing urea, or deficient in vitamin A. However, lymphocytosis, heteropenia , and monocytosis were recorded in birds fed the high calcium diet.

De Duffard AME; Bortolozzi A; Olguin MC; et al. (1977) **Intoxication by non-protein nitrogen compounds in rat feed**. Drug Chem Toxicol 22: 421-433. White rats (Wistar origin) showed acute behavioral and physiol. changes followed by death in 70% of the animals that could be attributed to a new batch of laboratory rat pellets provided two weeks before. High levels of urea (260 mg/kg) and ammonia (540 mg/kg) were found in the feed while usual values in similar feed were 48 mg/kg and 82 mg/kg resp. Suspecting an ammonia intoxication, concns. of ammonia and urea were determined in blood, brain and liver. Brain neurotransmitters and blood tryptophan and serotonin (5-HT) were also determined Blood ammonia in rats fed the contaminated feed was about 100% higher than those fed the normal feed while liver and brain ammonia were three and four fold high resp. Liver and brain urea were four to five fold and about 100% higher in the exposed group than in the group fed the control diet, resp. Blood 5-HT increased 62.33% in females and 99% in males whereas brain 5-HT increased 83.13% in females and 70.47% in males. There was a 59.8% decrease in brain dopamine levels in females and a 38.65% decrease in males. Liver histol. showed small droplets of fat stores mainly in centrolobular hepatocytes. No differences in blood or liver cholesterol concns. were observed whereas liver triacylglycerides were significantly higher in intoxicated females. This study illustrates a problem of food borne intoxication that justifies the need for exhaustive analyses of unusual compds. in every feed batch; moreover, it is demonstrated that rat behavior appears to be the earliest biomarker of ammonia exposure.

Dierickx, PJ. (1989) **Cytotoxicity testing of 114 compounds by the determination of the protein content in Hep G2 cell cultures**. Toxicology in Vitro 3: 189-193. The cellular protein content measured in cultured Hep G2 cells was used as the endpoint for determining the cytotoxicity of a range of 114 chemical compds. The relative toxicity of the test compds. was quantified by the determination of the PI50, which is the concentration of xenobiotic required to produce a 50% reduction in protein content of the culture after 24 h. Surfactants and heavy metals consistently had low PI50 values. Hep G2 cells were very sensitive to compds. with more than one carboxyl group. Triacetin and glutathione were identified as false positives. Thus, the PI50 assay could be a useful pre-screening method to test for the cytotoxicity of chems.

dos Passos Lemos A; Peres-Sampaio CE; Guimaraes-Motta H; et al. (2000) **Effects of naturally occurring polyols and urea on mitochondrial F0F1ATPase**. Z Naturforsch [C] 55: 5-6. We show that urea inhibits the ATPase activity of MgATP submitochondrial particles (MgATP-SMP) with Ki = 0.7 M, probably as a result of direct interaction with the structure of F0F1-ATPase. Counteracting compounds (sorbitol, mannitol or inositol), despite slightly (10-20%) inhibiting the ATPase activity, also protect the F0F1-ATPase against denaturation by urea. However, this protection was only observed at low urea concentrations (less than 1.5 M), and in the presence of three polyols, the Ki for urea shift from 0.7 M to 1.2 M. Urea also increases the initial activation rate of latent MgATP-SMP in a dose-dependent-manner.

However, when the particles (0.5 mg/ml) were preincubated in the presence of 1 M, 2 M or 3 M urea, a decrease in the activation level occurred after 1 h, 30 and 10 min, respectively. At high MgATP-SMP concentration (3 mg/ml) a decrease in activation was observed after 2 h, 1 h and 20 min, respectively. These data indicate that the effect of urea on the activation of MgATP-SMP depends on time, urea and protein concentrations. It was also observed that polyols suppress the activation of latent MgATP-SMP in a dose-dependent manner, and protect the particles against urea denaturation during activation. We suppose that a decrease in membrane mobility promoted by interactions of polyols with phospholipids around the F0F1-ATPase may also increase the compactation of protein structure, explaining the inhibition of natural inhibitor protein of ATPase (IF1) release and the activation of the enzyme.

Esayan AM; Titova VA; Shishkina LI; et al. (1997) **Effect of urea on the course of experimental uremia in subtotally nephrectomized rats**. Patol Fiziol Eksp Ter 2: 39-41. Increased plasma levels of urea appear to have an important role in the progression of renal failure, as shown in Wistar rats with exptl. uremia induced by subtotal nephrectomy. Renal structural lesions and dysfunction were the same as in rats fed a high-protein diet and in those on a low-protein diet supplemented with urea. The changes were much less in the control group of rats fed a low-protein diet alone. The findings suggest that high blood urea concns. appear to be a cause of intraglomerular hemodynamic disturbances in renal failure.

Felipo V; Minana MD; Grisolia S. (1988) **Long-term ingestion of ammonium increases acetylglutamate and urea levels without affecting the amount of carbamoyl-phosphate synthase**. Eur J Biochem 176: 567-571. Rats were fed the following diets: standard (20% protein), high-protein (80%), protein-free, standard plus ammonium and protein-free plus ammonium for six weeks. The standard plus ammonium diet was prepared to contain ammonia equivalent to that supplied by the high-protein diet. Addition of ammonium acetate (20% by mass) to the 20% protein or protein-free diets results in 2.3- and 10-fold increases of urea excretion respectively, without increase of carbamoyl-phosphate synthase. Supplementation of the standard diet with ammonium increases the mitochondrial content of acetylglutamate from 830 to 1590 pmol/mg protein, and of the protein-free diet from 130 to 1040 pmol/mg. However, ingestion of ammonium did not increase the activity of acetylglutamate synthase. Therefore the efflux of acetylglutamate from mitochondria was determined. After 30 min at 37 degrees C liver mitochondria from rats on standard diet released 61% of the initial acetylglutamate while mitochondria from animals on standard plus ammonium diet released only 20%. These results indicate that ingestion of ammonium increases the content of acetylglutamate in rat liver by decreasing its efflux from mitochondria. This effect is similar to that produced in mice by a high protein diet [Morita et al. (1982) J. Biochem. (Tokyo) 91, 563-569]. However, while the high-protein diet increases carbamoylphosphate synthase content, the ammonium diet does not.

Fridovich I. (1964) **Competitive inhibition of xanthine oxidase by urea and guanidinium ion**. J Biol Chem 239: 3519-3521.

Gabbe E. (1926) **Effect of urea on the excitability and O consumption of muscle. Original Title: UBER DIE WIRKUNG DES HARN-STOFFS AUF ERREGBARKEIT UND SAUERSTOFFVERBRAUCH DER MUSKELN**. Physik Med Ges Wurzburg Verhandl 51: 111-115. The muscles of frogs injected with urea (3-10 gm. per kgm. body weight) show increased excitability to mechanical stimuli. Contraction is rapid and is sustained for 1-3 sec, followed by rapid relaxation. Faradic stimulation causes no alteration in excitability after injection of urea, but contraction is of the same type; its strength is sometimes increased, sometimes diminished. The action of urea in increasing contraction of isolated frog's heart points to the action being specific, and not due to osmotic changes. Sympathectomy increases metabolism after injection of small, and diminishes it after injection of large, doses of urea. Since sympathectomy also causes an accumulation of urea in muscles, it is concluded that

the general increase in metabolism following injection of urea may in part be due to an increase in muscular activity. ABSTRACT AUTHORS: W. J. Wadge

Gianfreda L; Marrucci G; Greco G Jr. (1986) **Deactivation of free and stabilized acid phosphatase by urea**. Biotechnol Bioeng 28: 1647-1652. Tests on acid phosphatase (E.C. 3.1.3.2) deactivation by urea have been performed at two pH values. Two conditions have been used; native enzyme operating batchwise in dilute solution and stabilized enzyme in continuous flow ultrafiltration membrane reactor. Stabilization is acheived by confining the enzyme within a concentrated solution of a linear chain polymer that forms a polarization layer over the membrane. The results provide significnat information on the kinetics and thermodynamics of the complex phenomena taking place during deactivation. Deactivation by urea is also compared with thermal deactivation.

Glinos AD; Bardi GN; Dermitzaki KC; et al. (1983) **Cytokinetic and cytotoxic effects of urea on HeLa cells in suspension cultures**. J Natl Cancer Inst 71: 1211-1219. Total and viable cell counts, differential mitotic cell counts, and incorporation of tritiated thymidine were used to study the kinetics of suspension cultures of HeLa cells exposed to urea concentrations of 0.5-1.5%. Aside from any nonspecific osmotic effects, urea concentrations of 1.0-1.4% exhibited significant cytokinetic and cytotoxic effects. Most characteristically, mitotic cells arrested in metaphase began to accumulate 4-6 hours after addition of urea and reached a peak at 15-18 hours. Thus when the cells were in the S-phase or at the S/G2 boundary at the time of addition of urea, they exhibited metaphase arrest. Subsequently, cultures continuously exposed to urea showed a decline in the mitotic index, indicating that the entry rate of cells into mitosis is lower than the rate at which cells escape from the mitotic block. Such cultures exhibited numerous abnormal and abortive mitoses and a decrease in growth and viability of the cell populations. In contrast to the initial single wave of arrested mitosis seen with continuous exposure to urea, intermittent exposure on alternate days resulted in successive waves of arrested metaphases and had considerably more pronounced effects on the growth and viability of the cultures.

Gough A. (2006) **Diagnosis of azotaemia: when does high urea not equal kidney disease?** Vet Times 36: 14-15.

Hansen BA; Krog B; Vilstrup H. (1986) **Insulin and glucose decreases the capacity of urea-N synthesis in the rat**. Scand J Clin Lab Invest 46: 599-603. The relationship between insulin concentration (32-980 mU/l) and the capacity of urea-N synthesis (CUNS) was investigated with alanine as nitrogen source in 26 nephrectomized rats. The blood glucose concentration was kept constant by the 'glucose clamp' technique, and the endocrine pancreatic response was controlled by somatostatin. The CUNS was determined as the accumulation of urea corrected for intestinal hydrolysis at a constant amino acid concentration within the interval 7.3-11.6 mmol/l. At insulin concentration above 200 mU/l CUNS was decreased from 10 to 6 mumol (min X 100 g body wt)-1. At lower insulin concentrations the decrease was proportional. Hyperglycaemia 14.8 mmol/l decreased CUNS to 6.3 mumol (min X 100 g body wt)-1. The basal rate of urea-N synthesis was reduced from 3.8 to 1.9 mumol (min X 100 g body wt)-1 by insulin concentrations above 200 mU/l. The estimated alanine elimination (5.8 mumol(min X 100 g body wt)-1) was unchanged by insulin and reduced to 3.3 mumol(min X 100 g body wt)-1) by hyperglycaemia. Somatostatin infusion had no effect on CUNS or alanine elimination. It is suggested that the capacity of urea-N synthesis is subject to short term regulation independently by insulin and glucose.

Hansen BA; Vilstrup H. (1985) **A method for determination of the capacity of urea synthesis in the rat**. Scand J Clin Lab Invest 45: 315-320. The relationship between total blood alpha-amino nitrogen concentration and urea synthesis rate was investigated with alanine as nitrogen source in 24 rats. Alanine was given as prime-continuous doses for 70 min so that constant amino acid concentration was attained

between 5.5 and 34 mmol/l. Urea synthesis rate was assessed as accumulation in body water, corrected for intestinal hydrolysis. There was a positive correlation between nitrogen balance and alpha-amino nitrogen concentration. Urea synthesis rate in relation to amino acid concentration suggested barrier-limited substrate inhibition kinetics and data were examined accordingly by non-linear regression analysis. The estimated kinetic constants (mean +/- standard deviation) were: Vmax: 19.2 +/- 3.3 mumol (min X 100 g BW)-1, Km: 1.74 +/- 0.5 mmol/l, Ki: 6.84 +/- 1.9 mmol/l, and the barrier: 5.4 +/- 0.13 mmol/l. Because of the substrate inhibition, saturation cannot be attained, but the maximum synthesis rate, i.e. the capacity of urea nitrogen synthesis (CUNS), can be measured within 95% of the theoretical maximum in the concentration interval 7.3-11.6 mmol/l. CUNS was 9.16 +/- 0.81 mumol (min X 100 g BW)-1 (mean +/- standard deviation). Substrate-independent regulation of urea synthesis, e.g. by changes in liver mass or hormonal concentration, can be studied by this measure.

Hendrikx A; Epstein FH. (1958) **Effect of feeding protein and urea on renal concentrating ability in the rat**. Am J Physiol 195: 539-542. Maximum urinary concentration, achieved after dehydration or after administration of vasopressin, was significantly higher in rats prefed for 3 days on a 40% protein diet, or a 10% protein, 10% urea diet, than in the same rats fed an isocaloric diet containing 10% protein. The effect of protein in enhancing renal concentrating ability was slightly but significantly greater than that of an isonitrogenous quantity of urea. The solute concentration of urine excreted after large amounts of exogenous vasopressin was not as high as that reached after dehydration alone. ABSTRACT AUTHORS: Auth. abst

Horiike K; Merrill AH Jr.; McCormick DB. (1979) **Activation and inactivation of rabbit liver pyridoxamine (pyridoxine) 5'-phosphate oxidase activity by urea and other solutes**. Arch Biochem Biophys 195: 325-335.

Kaladze T; Oniani J; Kudryashov A; et al. (2002) **Implementation of algologic tests in the rapid assessment of toxic-genetic properties of xenobiotics**. Sakartvelos Mecnierebata Akademiis Macne, Biologiis Seria [continued by Izv Akad Gruz Ssr. Ser Biol] 28: 315-323. The work deals with the methods for evaluation of toxic-genetic effects, with Chlorella vulgaris and Nitella flexilis cells. With an example of joint action of phenol and formaldehyde, feasibility and effectiveness of such anal. has been shown. According to the results of electroalgol. anal. a classification scheme is proposed for the xenobiotics according to the toxicity.

Kamm DE; Wu L; Kuchmy BL. (1987) **Contribution of the urea appearance rate to diuretic-induced azotemia in the rat**. Kidney Int 32: 47-56. Studies were performed to evaluate the contribution of the urea appearance rate to the elevated plasma urea concentration found during diuretic-induced sodium depletion. Negative sodium balance of -1162 + 29 microEq/100 g body wt was induced over a four day period by the administration of furosemide, 20 to 30 mg/ kg/d i.p., to rats ingesting a sodium free diet. When compared with sodium replete controls, sodium depletion significantly increased the plasma urea concentration (65.0 +/- 3.1 vs. 26.4 +/- 1.1 mg/dl) through both an increase in the urea appearance rate (160 +/- 5.2 vs. 125 +/- 3.5 mg/day/100 g body wt), and a decrease in the urea clearance rate (1.99 +/- 0.14 vs. 3.16 +/- 0.12 ml/min/kg). The urea appearance rate increased on the first day of diuretic administration, remained elevated three days after stopping diuretics, rapidly returned to control levels after sodium repletion, and was significantly correlated with the magnitude of sodium deficit. Similar results were obtained when diuretic-induced sodium depletion was produced in adrenalectomized animals. After four days of sodium depletion the plasma concentration was increased for some amino acids but not for the plasma total amino acid, nitrogen concentration. The results indicate that sodium depletion increases the urea appearance rate through a mechanism that is independent of adrenal function. Thirty to sixty percent of the elevation in plasma urea concentration that occurs in the rat during

diuretic-induced sodium depletion can be accounted for by an enhanced urea appearance rate.

Kauker ML; Lassiter WE; Gottschalk CW. (1970) **Micropuncture study of effects of urea infusion in tubular reabsorption in the rat**. Am J Physiol 219: 45-50.

Kojima H; Katsura E; Takeuchi S; et al. (2004) **Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells**. Environ Health Perspect 112: 524-531. The authors tested 200 pesticides, including some of their isomers and metabolites, for agonism and antagonism to two human estrogen receptor (hER) subtypes, hER? and hER?, and a human androgen receptor (hAR) by highly sensitive transactivation assays using Chinese hamster ovary cells. The test compds. were classified into nine groups: organochlorines, di-Ph ethers, organophosphorus pesticides, pyrethroids, carbamates, acid amides, triazines, ureas, and others. These pesticides were tested at concns. < 10-5 M. Of the 200 pesticides tested, 47 and 33 showed hER?- and hER?-mediated estrogenic activities, resp. Among them, 29 pesticides had both hER? and hER? agonistic activities, and the effects of the organochlorine insecticides ?-benzene hexachloride (BHC) and ?-BHC and the carbamate insecticide methiocarb were predominantly hER? rather than hER? agonistic. Weak antagonistic effects toward hER? and hER? were shown in five and two pesticides, resp. On the other hand, none of tested pesticides showed hAR-mediated androgenic activity, but 66 of 200 pesticides exhibited inhibitory activity against the transcriptional activity induced by 5?-dihydrotestosterone. In particular, the antiandrogenic activities of two di-Ph ether herbicides, chlornitrofen and chlomethoxyfen, were higher than those of vinclozolin and p,p'-dichlorodiphenyl dichloroethylene, known AR antagonists. The results of our ER and AR assays show that 34 pesticides possessed both estrogenic and antiandrogenic activities, indicating pleiotropic effects on hER and hAR. The authors also discussed chemical structures related to these activities. Taken together, our findings suggest that a variety of pesticides have estrogenic and/or antiandrogenic potential via ER and/or AR, and that numerous other manmade chems. may also possess such estrogenic and antiandrogenic activities.

Kraenzlin B; Schieren G; Gretz N. (1997) **Azotemia and extrarenal manifestations in old female Han:SPRD (cy/+) rats**. Kidney Int 51: 1160-1169. In humans suffering from polycystic kidney disease (PKD) a gender difference is seen with males exhibiting a faster rate of progression of chronic renal failure than females. The aim of this study was to examine renal function in female rats suffering from autosomal dominant PKD [Han:SPRD (cy/+)] and to look for the occurrence of extrarenal organ manifestations of PKD. In young (2 mo) as well as in old female rats (21 mo) relative kidney weight was greater in affected than unaffected animals. In contrast, only the old affected female rats developed azotemia (serum urea 95 mg/dL) and severe cystic kidney transformation. Furthermore, old affected female rats exhibited liver cysts (affected 42%; unaffected 3%) and pancreatic cysts (affected 69%; unaffected 15%). Liver cyst epithelia stained pos. for cytokeratin 19, a marker for bile duct epithelia. By immunohistochem. liver cysts exhibited a similar extracellular matrix composition as observed in renal cysts of the same animals (staining pos. for laminin, fibronectin and heparan sulfate proteoglycan, but not collagen I). This study proves PKD in the Han:SPRD (cy/+) rat model to be a truly multiorgan disease with a close resemblance of the human disease.

Liapina LA; Kudriashov BA. (1975) **[Heparin-urea complex and its biological properties]**. Nauchnye Doki Vyss Shkoly Biol Nauki 10: 46-49.

Lin JK; Chen YC; Horng SC; et al. (1973) **Inhibition of urea on the hepatocarcinogensis induced by N, N-dimethyl-4-aminoazobenzene in rats**. Taiwan Yi Xue Hui Za Zhi 72: 262-266.

Litvinova L; Viru A. (1995) **Effect of exercise and adrenal insufficiency on urea production in rats**.

Eur J Appl Physiol Occup Physiol 70: 536-540. Experiments on Wistar rats were designed to study the effect of exercise on urea production in the liver of intact and adrenalectomized rats. The urea production rate was assessed by the 14C-urea content in liver tissue after administration of NaH14CO3. In intact rats swimming caused increases in 14C-urea content in the liver compared to the resting concentrations in intact control rats: by 45% after 30 min of swimming carrying an additional load of 10% body mass by, 35% after 3 h of swimming without an additional load and by 103% after 10 h of swimming. Concentrations of urea in liver and blood were elevated simultaneously. The specific activity of 14C-urea did not change significantly as a result of the exercise performed. In adrenalectomized rats the basal rate of urea production was reduced by an insignificant amount, but swimming for 3 h resulted in a decrease in liver 14C-urea (by 24%). The results confirmed the exercise-induced increase in urea production and indicated as essential role for adrenal hormones in this response.

Litwack G; Sears-Gessel M; Winicov I. (1966) **Inhibition of tyrosine aminotransferase activity by low concentrations of urea**. Biochim Biophys Acta 118: 351-362. Urea and guanidine hydrochloride in relatively low concentrations can completely inhibit tyrosine aminotransferase (L-tyrosine:2-oxo-glutarate aminotransferase, EC 2.6.1.5) activity by direct addition of the inhibitor to the assay system of prior incubation systems. Guanidine hydrochloride is more effective on a molar basis than urea; however, the action of these reagents is qualitatively similar. The kinetic mode of urea inhibition in the assay is reversible with respect to enzyme and is noncompetitive with respect to each reactant of the system. Simultaneous addition of about 10-4 [image] pyridoxal phosphate or pyri-doxamine phosphate to the prior incubation system affords nearly complete protection from concentrations of urea or guanidine hydrochloride which would produce 50% inhibition in the subsequent assay. L- Tyrosine, [alpha]-ketoglutarate, pyridoxal hydrochloride, or pyridox-amine hydrochloride do not protect from urea inhibition under these conditions. The fact that protection from urea inactivation requires concentrations of coenzyme of about 100 x Km together with the non-competitive mode of inhibition suggests that urea induces structural changes. The inhibition resulting from the presence of urea with enzyme in prior incubation systems is more extensive when Tris buffer is used in prior incubation than when phosphate buffer is used, although Tris buffer does not significantly inhibit enzymatic activity compared to phosphate buffer in the assay systems. This protective effect of phosphate ion can be imitated by arsenate ion in comparison to Tris buffer in the prior incubation system. Anionic surface active agents such as sodium dodecylsuliate or sodium deoxycholate are also very effective inhibitors of enzymatic activity, whereas a nonionic detergent was not inhibitory. The inhibition by anionic surface active agents is not eliminated by coenzyme in the prior incubation system and therefore appears to be qualitatively different from the action of urea or guanidine hydrochloride. ABSTRACT AUTHORS: Authors

Lukash AI; Kartashev IP; Antipina TV. (1980) **Inhibition of lipid per oxidation in tissues by urea**. Izv Sev Kavk Nauchn Tsentra Vyssh Shk Estestv Nauki: 102-105. The redox-potential of bivalent Fe after oxidation in the presence of urea was studied in model systems. The correlational interrelationships between urea levels and peroxidation products in tissues were also studied. A solution of iron sulfate with and without urea and a citrate buffer solution of pH 5.8 were used in the model systems. In the in vivo experiment white rats were subjected to peritoneal introduction of urea in aqueous solution at a rate of 200 mg/100 g of rat weight. The maximum protective effect was obtained with this concentration. The content of malonic dialdehyde was determined in the rat brain, liver and lungs. The maximum increase in urea after injection was 190% in the brain, 269% in the liver and 360% in the lungs. The most rapid rate of urea removal from tissues was from the liver followed by the lungs and then the brain. The possible regulation of peroxidation processes by urea was demonstrated. The probable mechanism of antioxidative action of urea was its inhibition of the oxidation of bivalent Fe and the production of free radicals initiating peroxidation chains during this process.

March BE; Biely J. (1971) **Urea tolerance in growing and adult chickens**. Poult Sci 50: 1077-1080.

Mashino T; Fridovich I. (1987) **Effects of urea and trimethylamine-N-oxide on enzyme activity and stability**. Arch Biochem Biophys 258: 356-360. The interactions of urea, trimethylamine-N-oxide (TMAO), and related solutes on a number of enzymes were examined. Urea inhibited enzymatic activity and accelerated the thermal inactivation of catalase, whereas TMAO activated some enzymes but inhibited others. The effects of urea and of TMAO, whether parallel or in opposition, were exerted independently. Thus, in those cases where TMAO increases enzymatic activity, it did so to the same relative degree, whether or not urea was present. TMAO markedly decreased the rate of thermal inactivation of catalase, indicating that it does favor compact protein structures. The assumption that TMAO factors compaction of protein structure, whereas urea has the contrary effect, does not lead to the expectation that TMAO must always oppose the effect of urea on enzymatic activity, since the most compact form of an enzyme may not always be the most active form.

Medaille C; Trumel C; Concordet D; et al. (2004) **Comparison of plasma/serum urea and creatinine concentrations in the dog: a 5-year retrospective study in a commercial veterinary clinical pathology laboratory**. J Vet Med A Physiol Pathol Clin Med 51: 119-123. Serum/plasma urea (S/P-urea) and creatinine (S/P-creatinine) concentrations are routinely assayed as indirect markers of glomerular filtration rate and have been reported to be highly correlated. The aim of this study was to evaluate the relationship between the two analytes in a large number of unselected samples submitted to a commercial laboratory. In 4799 pairs of results, the correlation was high (r = 0.795) and was not affected by sex or age. The relationship between the two analytes was best represented by a second-order polynomial equation. However, the dispersion of results was large and there was a high percentage of cases (27.5%) where S/P-urea was increased and S/P-creatinine normal (< or =120 micromol/l for this laboratory), while there was a low percentage of cases where S/P-creatinine (1.6%) was increased and S/P-urea normal (< or =8 mmol/l for this laboratory). The discrepancy between increases in S/P-urea and S/P-creatinine might not only reflect a high incidence of non-renal factors of variation for S/P-urea but also an effect of the size or muscle mass of the dogs on the limit of decision for S/P-creatinine. In dogs with normal S/P-urea, there was a significant effect of the size on the 0.975 quantile of S/P-creatinine, ranging from 106 micromol/l in very small dogs to 133 micromol/l in large and very large dogs. This study shows that isolated increases in S/P-urea could be misleading for the diagnosis of renal diseases and that the reference intervals of S/P-creatinine should be re-evaluated according to breed or muscle mass of dogs.

Mihich E; Simpson CL; Loth L; et al. (1969) **Toxic and patologic effects of 4,4'-diacetyl-diphenyl-urea-bis(guanylhydrazone), a new antileukemic agent**. Cancer Res 29: 1056-1061.

Mincea M; Lacrama A-M; Ostafe V. (2004) **Use of bovine liver alkaline phosphatase for testing at molecular level the toxicity of chemical compounds**. Ann West Univ Timisoara Ser Chem 13: 87-98. Especially in the case of new chems., their effects on environment, human health and live quality has to be assessed. As a part of a strategy implying multidimensional risk anal., an ecotoxicol. test battery was designed. Part of this systemic methodol. is the use of multienzymic kit for testing the effect of chemical compound at mol. level on several enzymes. The requirements for these enzymes and a model of how the enzyme has to be selected is presented. Alkaline phosphatase from calf liver fulfill many of these requirements: it is found in almost all organisms, it participate in major biochem. pathways, it is easy to extract and to analyze and has many known competitive and uncompetitive inhibitors, that can be used as model for testing the effects of new chems. The enzyme exhibit an optimum pH at 10.5 in glycine buffer, has a specific activity of 1500 nmol p-nitrophenyl phosphate(min-1(g-1 calf liver. The apparent KM for p-nitrophenyl phosphate was 0.166 mM. The use of enzyme for testing the effect of chems. was

exemplified for EDTA, CuCl2, Phenylalanine and urea.

Moeslinger T; Friedl R; Volf I; et al. (1999) **Urea induces macrophage proliferation by inhibition of inducible nitric oxide synthesis**. Kidney Int 56: 581-588. Background. Atherosclerosis is a major cause of morbidity and mortality in chronic renal failure and is associated with the proliferation of macrophages within atherosclerotic lesions. Methods. Because the progression of atherosclerosis as a consequence of decreased nitric oxide synthesis has been described, we investigated the correlation between the inhibition of inducible nitric oxide synthase (iNOS) by urea, macrophage proliferation as assayed by cell counting, tritiated thymidine incorporation and measurement of cell protein, and macrophage apoptosis. Results. Urea induces a dose- dependent inhibition of inducible nitric oxide synthesis in lipopolysaccharide-stimulated mouse macrophages (RAW 264.7) with concomitant macrophage proliferation. Macrophage proliferation, as determined by cell counting, became statistically significant at 60 mM urea, corresponding to a blood urea nitrogen level of 180 mg/100 ml, concentrations seen in uremic patients. iNOS protein expression showed a dose-dependent reduction, as revealed by immunoblotting when cells were incubated with increasing amounts of urea. The decrease of cytosolic DNA fragments in stimulated macrophages incubated with urea shows that the proliferative actions of urea are associated with a decrease of NO-induced apoptosis. Conclusions. Our data demonstrate that the inhibition of iNOS-dependent NO production caused by urea enhances macrophage proliferation as a consequence of diminished NO- mediated apoptosis.

Morgan DB; Payne RB. (1979) **Laboratory test for kidney function--urea or creatinine**. Lancet 2: 1014.

Nakamura K; Murai K; Nakatsuji K; et al. (1968) **Neuropharmacological and toxicological studies on a new anti-epileptic, N-alpha-ethyl-phenylacetyl-N'-acetyl urea in experimental animals**. Arzneimittelforschung 18: 524-529.

Nauss KM; Locniskar M; Sondergaard D; et al. (1984) **Lack of effect of dietary fat on N-nitrosomethyl urea (NMU)-induced colon tumorigenesis in rats**. Carcinogenesis 5: 255-260. The effect of alterations in the quality and quantity of dietary fat on N-nitrosomethyl urea (NMU)-induced colon cancer in rats was studied. Weanling Sprague-Dawley rats were fed semipurified diets containing 24% beef fat, 24% corn oil, 24% Crisco or the three fats in equal parts to make a total of 5% fat. Macronutrients and micronutrients were adjusted to balance the nutrient to calorie ratios. After 4 weeks of dietary treatment, all rats, except vehicle-treated animals received NMU (1.5 mg) via intrarectal instillation, twice a week for 2 weeks. The animals continued receiving the experimental diets until intestinal tumors developed and surviving animals were sacrificed at 43 weeks. There was no effect of any of the high fat diets tested on intestinal tumor incidence, latency, distribution or size. Cumulative probability of death with colon carcinoma did not differ significantly among the dietary groups.

Neuhofer W; Fraek M-L; Beck FX. (2002) **Heat shock protein 72, a chaperone abundant in renal papilla, counteracts urea-mediated inhibition of enzymes**. Pfluegers Arch 445: 67-73. Urea, at concentrations routinely observed in the renal inner medulla during antidiuresis in many mammals, is a potent protein destabilizing agent that reduces the activity of many enzymes. The molecular chaperone heat shock protein 72 (HSP72) is expressed at about 5 ng/mug protein in the renal papilla and is thus 40 times more abundant than in the isosmotic cortex and may counteract the deleterious effects of high urea concentrations in the inner medulla. To test this hypothesis, we examined the effect of recombinant HSP72 on lactate dehydrogenase activity in the presence of 0.8 M urea in a cell-free system. The urea-induced increase in Km was reduced by 85% in the presence of 1 muM HSP72 but only by 6% by 100 mM betaine, a "counteracting" trimethylamine osmolyte. Conversely, the decrease in Vmax with 0.8 M

urea was not affected by HSP72 but was attenuated by 42% in the presence of betaine. The protective effect of HSP72 was confirmed by the attenuation of the urea-induced decrease in the activity of another model enzyme, beta-galactosidase, by lysate of HSP72-overexpressing MDCK cells. Hence, in addition to the trimethylamine osmolytes, HSP72 may participate in counteracting urea-mediated effects on protein function in the renal papilla.

Neurotox

Nikezic G; Horvat A; Nedeljkovic N; et al. (1998) **Influence of pyridine and urea on the rat brain ATPase activity**. Gen Physiol Biophys 17: 15-23. The neurotoxicity of pyridine and urea was investigated in respect to their ability to alter the activity of synaptosomal membrane Na+/K(+)-ATPase and Mg(2+)-ATPase. In vitro treatment with pyridine and urea stimulated Na+/K(+)-ATPase activity in a dose -dependent manner up to 40% and 60%, respectively. Mg(2+)-ATPase activity increased up to 40% after pyridine treatment, while urea had no effect at all. The neuroactive potencies of pyridine and urea were evaluated by estimating parameters Km and delta Vmax for enzyme stimulation, as well as Hill coefficient to estimate the levels of cooperativity for pyridine and urea binding. The results suggest that pyridine stimulates both enzymes, probably by interacting with some neuronal membrane components, and altering the lipid micro-environment of the ATPases. In contrast, urea stimulates the Na+/K(+)-ATPase only, assumingly by acting on it directly or via some other regulatory mechanism. Stimulation of Na+/K(+)-ATPase and Mg(2+)-ATPase by the substances tested and subsequent alteration of neuronal cell functioning could contribute to the CNS dysfunction upon chronic exposure to pyridine and urea.

Pankow D. (1968) **[Effect of urea on potassium chloride poisoning in mice]. EINFLUSS DES HARNSTOFFS AUF DIE KALIUMCHLORIDVERGIFTUNG BEI MAUSEN**. Klin Wochenschr 46: 0023-2173.

Pipitone V; Russo R. (1959) **Studies in in vitro and in vivo on the action of urea and phenol on the activity of lysozyme [English summ.]. Original Title: STUDIO SULL'INFLUENZA DELL'UREA E DEL FENOLO SULL'ATTIVITA ENZIMATICA DEL LISOZIMA. RICERCHE "IN VIVO" ED "IN VITRO" [English summ.]**. Boll Ist Sieroterap Milan 38: 391-399. In subjects with chronic nephritis and hyperazotemia, the authors noted a high lysozyme activity of serum, possibly due to the presence of activators. After study on the action of urea and phenol on lysozyme and Micrococcus lysodeicticus they excluded these 2 substances as possible activators, as neither influenced lysozyme activity nor lysed M. lysodeicticus. ABSTRACT AUTHORS: MDS

Pontieri GM; Ciccimarra F. (1962) **Inhibiting action of urea on the components of complement**. Giorn Microbiol 10: 129-150. Under investigation were the in vitro action of urea on complement from normal and uremic animals (guinea pigs and rabbits the serum of which is respectively rich and poor of C'activity); the fate of complement in animals where a uremic state was surgically induced by ligature of the ureters and; the action of exogenous urea on complement from the animals with increased levels of blood endogenous urea. The increase in the levels of endogenous urea nitrogen up to 5% does not influence the activity of C' and its components. The addition in vitro of exogenous urea to serum of either normal or uremic animals is followed by decrease of C' activity only when urea is in concentration higher than 2 [image]. Among the components of complement, C'4 is the most sensitive to urea, followed by C'2 and C'1. Almost resistant is C'3. No difference was found in the sensitivity to urea of the C' components in the serum of uremic animals. Rabbit C' is more resistant than guinea pig C' to inhibition by exogenous urea. The high sensitivity of C'4 to exogenous urea is interpreted as being due to the fact that C'4 is also very sensitive to the low pH. Urea and low pH have a very similar denaturing activity. Therefore it seems that the lowering of C' activity which takes place in serum of patients suffering from renal disorders

should not be correlated to the increased levels of endogenous urea.

Radhakrishnan TM; Russo SF; Walsh KA; et al. (1969) **The inhibition of trypsinogen activation by low concentrations of urea**. Arch Biochem Biophys 130: 326-331.

Roselli RJ; Rutledge D; Harris TR. (1985) **Interpretation of urea indicator curves from dog lungs: Comparison of heterogeneous and homogeneous flow models**. Fed Proc 44: 8745.

Sadowski J; Potvliege PR; Gepts W; et al. (1978) **Urea-induced proteinuria: a new experimental model**. Kidney Int 13: 253-256. In search of a rapid and reliable method of inducing proteinuria in vivo, we tested the action of concentrated urea in acute experiments in the dog. The proposed model of experimental glomerular lesion has certain potential advantages in pathophysiological and clinical investigation. It offers a unique possibility to study proteinuria in the dog, the species in which previous attempts to produce experimental nephrotic syndrome using the aminonucleoside of puromycin were not successful. The lesion is obtained rapidly and is easily reproducible. Since it is unilateral, the contralateral kidney, when subjected to severe ischemia, can be employed as a useful control. Further functional and morphologic studies would be required to explore more thoroughly various aspects of urea-induced proteinuria.

Sies H; Summer KH; Bucher T. (1975) **A process requiring mitochondrial NADPH: urea formation from ammonia**. FEBS Lett 54: 274-278.

Silva IV; Caruso-Neves C; Azeredo IM; et al. (2002) **Urea inhibition of renal (NA+ + K+)ATPase activity is reversed by cAMP**. Arch Biochem Biophys 406: 183-189. In the present work we studied the modulation of the effect of urea on the renal (Na+ + K+)ATPase by cAMP. We observed that urea inhibits the (NA+ + K+)ATPase activity in a dose -dependent manner, reaching 60% of inhibition at the concentration of 1M. This effect was completely reversed by dibutyryl-cAMP (dBcAMP) at 5 x 10(-4)M. The effect of dBcAMP was mimicked by 50 units of the catalytic subunit of protein kinase A and completely abolished by 5 x 10(-7)M H89, an inhibitor of protein kinase A. Addition of 1M urea decreases basal phosphorylation of the immunoprecipitated (NA+ + K+)ATPase in 50%, with this effect completely reversed by 5 x 10(-4)M dBcAMP. Furthermore, 5 x 10(-4)M dBcAMP by itself induced (NA+ + K+)ATPase phosphorylation. Taken together these data indicate that cAMP could be, in addition to the organic solutes already known, an important physiological modulator of the deleterious effect of urea on enzyme activity.

Sosinski J; Chapin C; Thakar JH; et al. (1991) **Influence of extracellular pH on the accumulation and cytotoxicity of N-(4-methylphenylsulfonyl)-N'-(4-chlorophenyl)urea in human cell lines**. Cancer Commun 3: 373-381. The effect of extracellular pH (pH(e)) on the accumulation and cytotoxicity of the diarylsulfonylurea antitumor agent N-(4-methylphenylsulfonyl)-N'-(4-chlorophenyl)urea (MPCU) has been examined. In a human colon adenocarcinoma cell line, GC3/C1, the initial rate of uptake of [3H]MPCU (2.4 microM) was increased by 4.5-fold as pH(e) was reduced from 7.4 to 6.5. Steady state levels of MPCU were inversely proportional to pH(e) and were 5-fold greater at pH 6.0 compared to 7.4. Similar results were obtained using Rh30 cells derived from an alveolar rhabdomyosarcoma. MPCU rapidly re-equilibrated after achieving steady state when pH(e) was altered, indicating that MPCU was not tightly bound within cells. In both cell lines, the uncoupling agent, carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP), significantly reduced (GC3/C1) or completely inhibited (Rh30) accumulation of MPCU at each pH(e) examined. Sodium azide had the same effect on the accumulation of MPCU as FCCP. The effects of FCCP and azide appeared to be due to collapse of the pH differential across the mitochondrial inner membrane rather than the gradient across the plasma

membrane. As extracellular pH (pH(e)) decreased, intracellular pH(pH(i)) also decreased in GC3/C1 cells, such that the greatest pH differential (pH(i) - pH(e)) was 0.2 units at pH(e) 6.0. Neither FCCP nor azide significantly altered this pH gradient, indicating a minor role, if any, for the plasma membrane pH gradient in accumulation of MPCU in GC3/C1 cells. The effect of pH(e) (7.4 to 6.0) on cytotoxicity of MPCU was determined after exposure of cells for 4 hr to various concentrations of MPCU in the presence of 10% fetal bovine serum. Decreasing the pH(e) from 7.4 to 6.0 increased the potency of MPCU by 4.7- and 4.5-fold in Rh30 and GC3/C1 cells, respectively. In cells exposed to drug/pH(e) combinations that resulted in 50% reduction in colony forming potential, the steady state levels of [3H]MPCU were similar (range 8.8 +/- 0.9 to 10.56 +/- 0.6 nmol/10(6) cells). These results demonstrate that decrease of pH(e) significantly enhanced the uptake of MPCU accumulation into an FCCP/azide-sensitive compartment, and cytotoxicity of this agent. These data further support the hypothesis that sequestration of diarylsulfonylureas into the FCCP/azide-sensitive compartment (probably mitochondria) was associated with its cytotoxicity. The role of pH(e) in determining therapeutic selectivity of diarylsulfonylureas is discussed.

Tahin QS; Paiva AC. (1970) **Effect of urea on the peptic proteolysis of ovalbumin**. Enzymologia 39: 284-288.

Takeuchi K; Ohuchi T; Harada H; et al. (1995) **Irritant and protective action of urea-urease ammonia in rat gastric mucosa. Different effects of ammonia and ammonium ion**. Dig Dis Sci 40: 274-281. The effects of urea-urease-ammonia on the rat gastric mucosa were examined and compared with those of NH4OH and NH4Cl. The mucosal application of urea with urease produced a reduction in potential difference (PD) in a dose-related manner for urea, and a significant drop was observed by > 0.1% urea in the presence of 100 units urease. Such PD reduction was also observed when the mucosa was exposed to either NH4OH (> 0.03%) or NH4Cl (> 1%); delta PD (20 mV) caused by 0.3% NH4OH and 3% NH4Cl was equivalent to that induced by 0.5% urea+urease (100 units). The combined oral administration of urea (approximately 6%) and urease (100 units) did not induce any macroscopic damage in the gastric mucosa. NH4Cl given orally had no or little effect on the mucosa at any dose levels even at 10%, while NH4OH given orally caused hemorrhagic lesions in the mucosa at the dose of > 0.3%. In contrast, both urea+urease and NH4Cl given prior to HCl/ethanol protected the gastric mucosa against damage in a dose-related manner, and a significant effect was obtained by urea at > 0.5% and by NH4Cl at > 1%. NH4OH was also effective in reducing the severity of HCl/ethanol-induced gastric lesions at lower dose (0.3%). The protective effect of urea+urease was attenuated significantly by prior administration of indomethacin or coadministration of hydroxyurea, while that of NH4Cl or NH4OH was mitigated by indomethacin.(ABSTRACT TRUNCATED AT 250 WORDS)

Tchounwou PB; Englande AJ Jr.; Malek EA. (1991) **Toxicity evaluation of ammonium sulphate and urea to three developmental stages of freshwater snails**. Arch Environ Contam Toxicol 21: 359-364. Studies were performed to evaluate the toxic effects of ammonium sulphate and urea (chemical fertilizers currently applied in ricelands of Cameroon) against eggs, juveniles, and adults of two species of freshwater snails (Helisoma trivolvis and Biomphalaria havanensis). Results obtained from ammonium sulphate tests indicated 24-h LC50 values of 558 mg/L and 669 mg/L for eggs; 393 mg/L and 526 mg/L for juveniles, and 701 mg/L and 657 mg/L for adults of H. trivolvis and B. havanensis, respectively. Similar analysis with urea revealed LC50 values of 14,241 mg/L and 13,532 mg/L for eggs; 18,255 mg/L and 24,504 mg/L for juveniles and 30,060 mg/L and 26,024 mg/L for adults of H. trivolvis and B. havanensis, respectively. Following 48 h exposure, the concentrations of ammonium sulphate killing 100% of snails were 1,250 mg/L and 1,000 mg/L for the adults of H. trivolvis and of B. havanensis, respectively. Those of urea were computed to be 25,000 mg/L for H. trivolvis and 35,000 mg/L for B. havanensis. In rice culture in Cameroon, these fertilizers are applied at doses of 100 kg/ha (ammonium

sulphate) and of 150 kg/ha (urea); hence, the above found concentrations lethal to snails appeared to be 10 to 13 times (ammonium sulphate) and to be 165 to 235 times (urea) higher assuming an average water depth of 10 cm in these ricefields. Therefore, the use of ammonium sulphate and urea as chemical fertilizers in ricelands of the Republic of Cameroon might adversely affect the survival of freshwater snails only in the case of spills or of stressful environmental conditions. Under normal laboratory conditions, both chemicals show a low molluscicidal activity with urea being about 25 to 35 times less potent than ammonium sulphate.

Temnialov ND; Abramets II. (1978) **[Nature of the sensitizing influence of urea on the effects of catecholamines]. O PRIRODE SENSIBILIZIRUIUSHCHEGO VLIIANIIA MOCHEVINY NA EFFEKTY KATEKHOLAMINOV**. Farmakol Toksikol 41: 296-298. Tests conducted on aortic strips of rabbits, portal veins and musculus rectococcygeus in rats showed the urea to nonspecifically increase the effects of norepinephrine at the expense of a greater calcium permeability of membranes and, possibly, also at the expense of a higher sensitivity of the myosin adenosine-triphosphatase to the action of calcium ions without affecting the function of adrenoreceptors.

Van Woert MH; Chung E; Yocca F. (1984) **Possible involvement of glycine in urea-induced myoclonus**. Abstr Soc Neurosci 10: 10-15.

Vanrenterghem Y; Vanholder R; Lammens-Verslijpe M; et al. (1979) **Sieving studies in 'urea-induced nephropathy' in the dog**. Clin Sci 58: 65-75. 1. The intrarenal infusion of concentrated urea after clamping of the renal artery produced immediate proteinuria in the dog. The predominant lesion on ultramicroscopy was destruction of the epithelial layer. Colloidal iron staining showed decreased fixed anionic charges in the capillary wall. 2. Sieving studies with neutral macromolecules such as 125I-labelled polyvinylpyrrolidone and [3H]dextran or an electronegatively charged polymer, [3H]dextran sulphate, showed a moderate increase in permeablility to the neutral tracers and a much more severe alteration of the lectrostatic barrier to the anionic polymer. The fractional clearance of dextran sulphate molecules increased to a greater extent than clearance of neutral dextrans of comparable size. 3. The shape of the curve relating the fractional clearance of dextran sulphate to molecular size is also modified in the contralateral kidney. This may be due to elevated plasma angiotensin II concentrations.

Varga ZS; Kakuk GY. (2000) **Urea-derived-cyanate affects oxidative burst in PMNLs by a concentration dependent manner**. Nephrol Dial Transplant 15: 17-20.

Vergnes HA; Grozdea JD. (1985) **A study of the selective inhibition by urea of neutrophil alkaline phosphatase isoenzymes**. Enzyme 34: 45-47. The kinetics of neutrophil alkaline phosphatase (AP) inactivation by urea were examined. In standardized conditions (molarity and pH of buffer, magnesium chloride and urea concentration), monitoring of the enzymatic reaction allows identification of neutrophil AP isoenzymes. This study confirms the heterogeneity of AP in normal human neutrophils.

Villarreal H; Ronces R; Sanchez V; et al. (1962) **Failure of L-arginine to protect in ammonia intoxication: its role in urea source**. Am J Physiol 202: 364-366.

Yang XY; Zhang Z; Cohen DM. (1999) **ERK activation by urea in the renal inner medullary mIMCD3 cell line**. Am J Physiol 277: F176-85. Urea- and NaCl-inducible extracellular signal-regulated kinase (ERK) phosphorylation exhibited dissimilar kinetics. Among cell lines examined, the effect of urea was unique to mIMCD3 inner medullary collecting duct cells and MDCK cells. Urea-inducible ERK activation was approximately 10-fold less sensitive to the MEK inhibitor, PD-98059, than was that of NaCl. This difference did not appear to be accounted for by differential activation of MEK isoforms.

Interestingly, the inhibitor of p38 activation, SB-203580, abrogated the effect of both urea and NaCl upon both ERK and MEK activation; however, the former was much less sensitive to the inhibitor. Consistent with this observation, NaCl was much more effective than urea at inducing p38 phosphorylation. The effect of hypertonic stress (e.g., sorbitol 100 mM) could be blocked by appropriate medium dilution such that isotonicity was maintained. In marked contrast, the effect of hyperosmotic urea could not be blocked in this fashion, implying the absence of dependence upon cell volume. Together, these data suggest that cells of the renal inner medulla are potentially uniquely responsive to urea and that urea and hypertonic stressors induce ERK activation through distinct mechanisms.

Zamboni P; Cortese I. (1965) **[Hypoglycemic action of large doses of urea]. AZIONE IPOGLICEMIZZANTE DELLE ALTE DOSI DI UREA**. Boll Soc Ital Biol Sper 41: 648-649.

Zhelyazkov D; Temnyalov N. (1990) **Endogenous urea(U): A candidate to be qualified as an autocoid (in vivo and in vitro data from animal and human studies)**. Eur J Pharmacology 183: 0014-2999

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

Al-Homrany, M. (2001) **In vitro effect of urea on serum enzymes levels**. Biomedical Research 12: 87-89. Urea is one of the waste products that accumulates in the body of the dialysis patients. The toxic effect of urea on the serum enzymes levels is studied in vitro. Urea did not change the activities of lactic dehydrogenase, creatine kinase, and transaminases, when added in vitro with a concentration of 300 mg/dL to the serum of the control group. However, the serum levels of gamma-glutamyl transpeptidase and alkaline phosphatase were found to be depressed (Z = -1.9917, P = 0.04 and Z = -1.9604, P = 0.05, resp.).

Bagnasco, SM. (2000) **How renal cells handle urea**. Cell Physiol Biochem 10: 379-384. The urine concentration process requires an osmolality gradient along the renal cortico-medullary axis, with highest values in the renal papilla. NaCl and urea are the major solutes in the renal inner medulla, concentrations of urea up to 500-600 mM are found in the rat renal papilla. Urea can diffuse across cell membranes and contributes to balance intracellular and extracellular osmotic equilibrium. However, urea has perturbing effects on enzyme activity, and in concentrations above 300 mM is toxic for renal cultured cells. There is increasing evidence that urea can induce cellular responses distinct from those due to NaCl and other non-permeable solutes, including upregulation of immediate-early genes (IEGs). Urea transport by epithelial and endothelial cells is important for intra-medullary urea recycling and preservation of high urea concentration in the inner medulla. Trans-cellular movement of urea in cells expressing urea transporters may influence intracellular levels of this solute and modulate urea-induced signaling pathways. Regulation of urea transporters expression and activity can therefore be viewed as one aspect of cellular adaptation to urea. We have identified tonicity-responsive transcription as one mechanism regulating expression of the urea transporter UT-A. The short-term and long-term effects of variable extracellular urea concentration on the function of renal cells remain still unclear.

Chung, E; Yocca, F; Van Woert, MH. (1985) **Urea-induced myoclonus: medullary glycine antagonism as mechanism of action**. Life Sci 36: 1051-1058. Stimulus sensitive myoclonus is a prominent symptom of uremia in both man and animals. Intravenous injection of urea into cats had been previously reported to produce spike and sharp wave electrical discharges in the medullary reticular

formation which correlated with the myoclonic movements. In the present investigations, intraperitoneal injections of 2 g/kg urea every 15 minutes for 4 injections produced myoclonus in rats accompanied by brain urea concentrations of 6.8 X 10(-2)M, which is sevenfold higher than normal. 10(-2) and 10(-1) M urea significantly reduced 3H-strychnine binding to rat medulla membranes by 30% and 43% respectively. Urea inhibition of 3H-strychnine binding was reversible and binding kinetics revealed that 10(-1)M urea decreased Bmax by 65% with no effect on the affinity. Brain glycine levels did not change after urea injections and urea had no effect on synaptosomal uptake of 3H-glycine. Urea did not alter 3H-GABA, 3H-glutamate and 3H-QNB receptor binding but decreased 3H-diazepam receptor binding in the medulla. Mannitol also reduced 3H-diazepam binding but had no effect on 3H-strychnine binding. Stereotaxic injection of the glycine receptor antagonist, strychnine, into the rat medullary reticular formation produced myoclonus, whereas Ro 15-1788, a benzodiazepine antagonist, had no effect. Urea may produce myoclonus by blockade of glycine receptors in the medullary reticular formation.

Cohen, DM; Yang, M; Zhang, Z. (1998) **Urea-inducible oxidative stress and Gadd153 induction**. Journal of the American Society of Nephrology, 9: 25-28.

D'Hooge, R; Van de Vijver, G; Van Bogaert, PP; et al. (2003) **Involvement of voltage- and ligand-gated Ca2+ channels in the neuroexcitatory and synergistic effects of putative uremic neurotoxins**. Kidney Int 63: 1764-1775. Renal failure has been viewed as a state of cellular calcium toxicity due to the retention of small fast-acting mols. The authors have tested this hypothesis and identified potentially neuroexcitatory compds. among a number of putative uremic neurotoxins by examining the acute in vitro effects of these compds. on cultured central neurons. The in vitro neuroexcitatory and synergistic effects of guanidinosuccinate and spermine were also examined in vivo. The acute effects of 17 candidate uremic neurotoxins on murine spinal cord neurons in primary dissociated cell culture were investigated using the tight-seal whole-cell recording technique. The compds. studied comprised low-mol.-weight solutes like urea, indoles, guanidino compds., polyamines, purines and phenoles, homocysteine, orotate, and myoinositol. Currents evoked by these compds. were further examined using various ligand- and voltage-gated ion channel blockers. The acute in vivo effects of guanidinosuccinate and spermine were behaviorally assessed following their injection in mice. It was shown that 3-indoxyl sulfate, guanidinosuccinate, spermine, and phenol evoked significant whole-cell currents. Inward whole-cell current evoked by 3-indoxyl sulfate was not blocked by any of the applied ligand- or voltage-gated ion channel blockers, and the compound appeared to influence miscellaneous membrane ionic conductances, probably involving voltage-gated Ca2+ channels as well. Phenol-evoked outward whole-cell currents were at least partly due to the activation of voltage-gated K+ channels, but may also involve a variety of other ionic conductances. Inward whole-cell currents evoked by guanidinosuccinate and spermine were shown to be due to specific interaction with voltage- and ligand-gated Ca2+ channels. Guanidinosuccinate-evoked current was caused by activation of N-methyl-D-aspartate (NMDA) receptor-associated ion channels. Low (?M) concns. of spermine potentiated guanidinosuccinate-evoked current through the action of spermine on the polyamine binding site of the NMDA receptor complex, whereas current evoked by high (mmol/L) concns. of spermine alone involved direct activation of voltage-gated Ca2+ channels. Finally, intracerebroventricular administration of 0.25 ?M spermine potentiated clonic convulsions induced by guanidinosuccinate. These neuroexcitatory and synergistic effects of guanidinosuccinate and spermine could take place at pathophysiol. concns. The observed in vitro and in vivo effects of uremic retention solutes suggest that the identified compds. could play a significant role in uremic pathophysiol. Some of the compds. tested displayed in vitro and in vivo neuroexcitatory effects that were mediated by ligand- and voltage-gated Ca2+ channels. The findings suggest a mechanism for the involvement of calcium toxicity in the central nervous system complications in renal failure with particular reference to guanidinosuccinate and spermine.

Kultz, D. (2004) **Hyperosmolality triggers oxidative damage in kidney cells**. Proc Natl Acad Sci USA 101: 9177-9178.

Kultz, D; Chakravarty, D. (2001) **Hyperosmolality in the form of elevated NaCl but not urea causes DNA damage in murine kidney cells**. Proc Natl Acad Sci USA 98: 1999-2004. This study demonstrates, by using neutral comet assay and pulsed field gel electrophoresis, that hyperosmotic stress causes DNA damage in the form of double strand breaks (dsb). Different solutes increase the rate of DNA dsb to different degrees at identical strengths of hyperosmolality. Hyperosmolality in the form of elevated NaCl (HNa) is most potent in this regard, whereas hyperosmolality in the form of elevated urea (HU) does not cause DNA dsb. The amount of DNA dsb increases significantly as early as 15 min after the onset of HNa. By using neutral comet and DNA ladder assays, we show that this rapid induction of DNA damage is not attributable to apoptosis. We demonstrate that renal inner medullary cells are able to efficiently repair hyperosmotic DNA damage within 48 h after exposure to hyperosmolality. DNA repair correlates with cell survival and is repressed by 25 microM LY294002, an inhibitor of DNA-activated protein kinases. These results strongly suggest that the hyperosmotic stress resistance of renal inner medullary cells is based not only on adaptations that protect cellular proteins from osmotic damage but, in addition, on adaptations that compensate DNA damage and maintain genomic integrity.

Miyata, Y; Asano, Y; Muto, S. (2002) **Hyperosmotic urea activates basolateral NHE in proximal tubule from P-gp null and wild-type mice**. Am J Physiol Renal Physiol 283: F771-783. Using the pH-sensitive fluorescent dye BCECF, we compared the effects of hyperosmotic urea on basolateral Na(+)/H(+) exchange (NHE) with those of hyperosmotic mannitol in isolated nonperfused proximal tubule S2 segments from mice lacking both the mdr1a and mdr1b genes (KO) and wild-type (WT) mice. All the experiments were performed in CO(2)/HCO-free HEPES solutions. Osmolality of the peritubular solution was raised from 300 to 500 mosmol/kgH(2)O by adding mannitol or urea. NHE activity was assessed by the Na(+)-dependent acid extrusion rate (J(H)) after an acid load with NH(4)Cl prepulse. In WT mice, hyperosmotic mannitol had no effect on J(H) at over the entire range of intracellular pH (pH(i)) studied (6.20-6.90), whereas in KO mice it increased J(H) at a pH(i) range of 6.20-6.45. In contrast, in both WT and KO mice, hyperosmotic urea increased J(H) at a pH(i) range of 6.20-6.90. In KO mice, J(H) in a hyperosmotic urea solution were similar to those in a hyperosmotic mannitol solution at a pH(i) range of 6.20-6.40 but were greater than in a hyperosmotic mannitol solution at a pH(i) range of 6.45-6.90. In WT mice, hyperosmotic urea caused an increase in V(max) without changing K(m) for peritubular Na(+). Staurosporine (the PKC inhibitor) inhibited hyperosmotic mannitol-induced NHE activation in KO mice, whereas it had no effect on hyperosmotic urea-induced NHE activation in WT or KO mice. Genistein (the tyrosine kinase inhibitor) inhibited hyperosmotic urea-induced NHE activation in WT and KO mice, whereas it caused no effect on hyperosmotic mannitol-induced NHE activation in KO mice. We conclude that hyperosmotic urea activates basolateral NHE via tyrosine kinase in tubules from both WT and KO mice, whereas hyperosmotic mannitol activates it via PKC only in tubules from KO mice.

Sakurada, H; Sato, A. (1927) **Detoxicating hormone of the liver. V. Prophylactic effect of yakriton against urea intoxication**. Tohoku Journal of Experimental Medicine 9: 66-69. cf. C. A. 21, 1141. Expts. with rabbits proved that yakriton is the regulating principle of urea as well as of ammonia. This fact gives addnl. support to the assumption that yakriton is the detoxicating hormone of the liver.

Wardle, EN. (1970) **A study of the effects of possible toxic metabolites of uraemia on red cell metabolism**. Acta Haematol 43: 129-143.

4.5.1 Role of urea transporters

Fenton, RA; Chou, CL; Sowersby, H; et al. (2006) **Gamble's "economy of water" revisited: studies in urea transporter knockout mice**. Am J Physiol Renal Physiol 291: F148-154. The Gamble phenomenon (initially described over 70 years ago as "an economy of water in renal function referable to urea") suggested that urea plays a special role in the urinary concentrating mechanism and that the concentrating mechanism depends in some complex way on an interaction between NaCl and urea. In this study, the role of collecting duct urea transporters in the Gamble phenomenon was investigated in wild-type mice and mice in which the inner medulla collecting duct (IMCD) facilitative urea transporters, UT-A1 and UT-A3, had been deleted (UT-A1/3-/- mice). The general features of the Gamble phenomenon were confirmed in wild-type mice, namely 1) the water requirement for the excretion of urea is less than for the excretion of an osmotically equivalent amount of NaCl; and 2) when fed various mixtures of urea and salt in the diet, less water is required for the excretion of the two substances together than the amount of water needed for the excretion of the two substances separately. In UT-A1/3-/- mice both of these elements of the phenomenon were absent, indicating that IMCD urea transporters play a central role in the Gamble phenomenon. A titration study in which wild-type mice were given progressively increasing amounts of urea showed that the ability of the kidney to reabsorb urea was saturable, resulting in osmotic diuresis above excretion rates of approximately 6,000 microosmol/day. In the same titration experiments, when increasing amounts of NaCl were added to the diet, mice were unable to increase urinary NaCl concentrations to >420 mM, resulting in osmotic diuresis at NaCl excretion rates of approximately 3,500 microosmol/day. Thus both urea and NaCl can induce osmotic diuresis when large amounts are given, supporting the conclusion that the decrease in water excretion with mixtures of urea and NaCl added to the diet (compared with pure NaCl or urea) is due to the separate abilities of urea and NaCl to induce osmotic diuresis, rather than to any specific interaction of urea transport and NaCl transport at an epithelial level.

Fenton, RA; Stewart, GS; Carpenter, B; et al. (2002) **Characterization of mouse urea transporters UT-A1 and UT-A2**. Am J Physiol Renal Physiol 283: F817-825. Specialized transporter proteins that are the products of two closely related genes, UT-A (Slc14a2) and UT-B (Slc14a1), modulate the movement of urea across cell membranes. The purpose of this study was to characterize the mouse variants of two major products of the UT-A gene, UT-A1 and UT-A2. Screening a mouse kidney inner medulla cDNA library yielded 4,047- and 2,876-bp cDNAs, the mouse homologues of UT-A1 and UT-A2. Northern blot analysis showed high levels of UT-A mRNAs in kidney medulla. UT-A transcripts were also present in testes, heart, brain, and liver. Immunoblots with an antiserum raised to the 19 COOH-terminal amino acids of rat UT-A1 (L194) identified immunoreactive proteins in kidney, testes, heart, brain, and liver and showed a complex pattern of differential expression. Relative to other tissues, kidney and brain had the highest levels of UT-A protein expression. In kidney sections, immunostaining with L194 revealed immunoreactive proteins in type 1 (short) and type 3 (long) thin descending limbs of the loop of Henle and in the middle and terminal inner medullary collecting ducts. Expression in Xenopus laevis oocytes showed that, characteristic of UT-A family members, the cDNAs encoded phloretin-inhibitable urea transporters. Acute application of PKA agonists (cAMP/forskolin/IBMX) caused a significant increase in UT-A1- and UT-A3-, but not UT-A2-mediated, urea transport.

Hu, M-C; Bankir, L; Michelet, S; et al. (2000) **Massive reduction of urea transporters in remnant kidney and brain of uremic rats**. Kidney Int 58: 1202-1210. Background. The facilitated urea transporters (UT), UT-A1, UT-A2, and UT-B1, are involved in intrarenal recycling of urea, an essential feature of the urinary concentrating mechanism, which is impaired in chronic renal failure (CRF). In this study, the expression of these UTs was examined in exptl. induced CRF. Methods. The abundance of mRNA was measured by Northern anal. and that of corresponding proteins by Western blotting in rats

one and five weeks after 5/6 nephrectomy (Nx). Results. At five weeks, urine output was enhanced threefold with a concomitant decrease in urine osmolality. The marked rise in plasma urea concentration and fall in urinary urea concentration resulted in a 30-fold decrease in the urine/plasma (U/P) urea concentration ratio, while the U/P osmoles ratio fell only fourfold. A dramatic decrease in mRNA abundance for the three UTs was observed, bringing their level at five weeks to 1/10th or less of control values. Immunoblotting showed complete disappearance of the 97 and 117 kDa bands of UT-A1, and considerable reduction of UT-A2 and UT-B1 in the renal medulla. Similar, but less intense, changes were observed at one-week post-Nx. In addition to the kidney, UT-B1 is also normally expressed in brain and testis. In the brain, its mRNA expression remained normal one-week post-Nx, but decreased to about 30% of normal at five-weeks post-Nx, whereas no change was seen in testis. Conclusions. (1) The decline in urinary concentrating ability seen in CRF is largely due to a major reduction of UTs involved in the process of urea concentration in the urine, while factors enabling the concentration of other solutes are less intensely affected. (2) The marked reduction of brain UT expression in CRF may be responsible for brain edema of dialysis disequil. syndrome observed in some patients after fast dialysis.

Inoue, H; Kozlowski, SD; Klein, JD; et al. (2005) **Regulated expression of renal and intestinal UT-B urea transporter in response to varying urea load**. Am J Physiol Renal Physiol 289: F451-458. Production, recycling, and elimination of urea are important to maintain nitrogen balance. Adaptation to varying loads of urea due to different protein intake or in renal failure may involve changes in urea transport and may possibly affect urea transporters. In this study, we examined the expression of the UT-B urea transporter in rats fed a low-protein diet (LPD), a high-protein diet (HPD), and a 20% urea-supplemented diet. In the kidney, UT-B protein abundance increased in the outer medulla of both LPD-fed rats and 20% urea-fed rats, without changes in the inner medulla of either group compared with controls. In HPD-fed rats, UT-B protein decreased significantly in both the outer and inner medulla. We identified expression of UT-B in the rat colon, as a 2-kb mRNA transcript and as an approximately 45-kDa protein, with apical localization in superficial colon epithelial cells. UT-B also is expressed in rat small intestine. In rat colon, UT-B protein abundance was mildly, but significantly, decreased in LPD-fed and 20% urea-fed rats. UT-B abundance also was examined in the colon of 7/8 nephrectomized, uremic rats and in HPD-fed rats and was not significantly different from that in control rats. These findings indicate that UT-B expression is regulated in response to different loads of urea, with a pattern that suggests involvement of tissue-specific regulatory mechanism in kidney and colon.

Kim, D; Klein, JD; Racine, S; et al. (2005) **Urea may regulate urea transporter protein abundance during osmotic diuresis**. Am J Physiol Renal Physiol 288: F188-197. Rats with diabetes mellitus have an increase in UT-A1 urea transporter protein abundance and absolute urea excretion, but the relative amount (percentage) of urea in total urinary solute is actually decreased due to the marked glucosuria. Urea-specific signaling pathways have been identified in mIMCD3 cells and renal medulla, suggesting the possibility that changes in the percentage or concentration of urea could be a factor that regulates UT-A1 abundance. In this study, we tested the hypothesis that an increase in a urinary solute other than urea would increase UT-A1 abundance, similar to diabetes mellitus, whereas an increase in urine urea would not. In both inner medullary base and tip, UT-A1 protein abundance increased during NaCl- or glucose-induced osmotic diuresis but not during urea-induced osmotic diuresis. Next, rats undergoing NaCl or glucose diuresis were given supplemental urea to increase the percentage of urine urea to control values. UT-A1 abundance did not increase in these urea-supplemented rats compared with control rats. Additionally, both UT-A2 and UT-B protein abundances in the outer medulla increased during urea-induced osmotic diuresis but not in NaCl or glucose diuresis. We conclude that during osmotic diuresis, UT-A1 abundance increases when the percentage of urea in total urinary solute is low and UT-A2 and UT-B abundances increase when the urea concentration in the medullary interstitium is high. These findings suggest that a reduction in urine or interstitial urea results in an increase in UT-A1 protein

abundance in an attempt to restore inner medullary interstitial urea and preserve urine-concentrating ability.

Lucien, N; Bruneval, P; Lasbennes, F; et al. (2005) **UT-B1 urea transporter is expressed along the urinary and gastrointestinal tracts of the mouse**. Am J Physiol Regul Integr Comp Physiol 288: R1046-1056. Selective transporters account for rapid urea transport across plasma membranes of several cell types. UT-B1 urea transporter is widely distributed in rat and human tissues. Because mice exhibit high urea turnover and are the preferred species for gene engineering, we have delineated UT-B1 tissue expression in murine tissues. A cDNA was cloned from BALB/c mouse kidney, encoding a polypeptide that differed from C57BL/6 mouse UT-B1 by one residue (Val-8-Ala). UT-B1 mRNA was detected by RT-PCR in brain, kidney, bladder, testis, lung, spleen, and digestive tract (liver, stomach, jejunum, colon). Northern blotting revealed seven UT-B1 transcripts in mouse tissues. Immunoblots identified a nonglycosylated UT-B1 protein of 29 kDa in most tissues and of 36 and 32 kDa in testis and liver, respectively. UT-B1 protein of gastrointestinal tract did not undergo N-glycosylation. Immunohistochemistry and in situ hybridization localized UT-B1 in urinary tract urothelium (papillary surface, ureter, bladder, and urethra), prominently on plasma membranes and restricted to the basolateral area in umbrella cells. UT-B1 was found in endothelial cells of descending vasa recta in kidney medulla and in astrocyte processes in brain. Dehydration induced by water deprivation for 2 days caused a tissue-specific decrease in UT-B1 abundance in the urinary bladder and the ureter.

Stewart, GS; Fenton, RA; Thevenod, F; et al. (2004) **Urea movement across mouse colonic plasma membranes is mediated by UT-A urea transporters**. Gastroenterology 126: 765-773. BACKGROUND & AIMS: Urea is a major nitrogen source for commensal bacteria that inhabit the large intestine. UT-A urea transporters mediate urea movement across plasma membranes. The aim of this study was to determine whether UT-A proteins are expressed in the mouse colon and, if so, whether they have a functional role in transcellular urea transport. METHODS: Mouse colonic UT-A transporters were investigated with Northern blot analysis, immunoblotting, immunolocalization, and refractive light flux experiments. RESULTS: Northern blot analysis showed that 4 UT-A transcripts were present in mouse colon. Two peptide-targeted polyclonal antibodies showed the presence of UT-A immunoreactive proteins in mouse colon. Antiserum ML446 targeted to the N-terminus of mouse UT-A1 detected proteins of 34 and 48 kilodaltons. Antiserum ML194 targeted to the C-terminus of mouse UT-A1 detected proteins of 48, 75, and 100 kilodaltons. Immunolocalization studies using ML446 showed the presence of UT-A proteins in cells throughout the colonic crypts. ML194 specifically stained cells located in the proliferative and stem regions of the lower portion of colonic crypts. Differential centrifugation and immunoblotting of colonic epithelia showed that UT-A proteins were present in plasma membrane-enriched fractions. Refractive light flux experiments using colonic plasma membrane vesicles showed a significant urea flux, which was completely inhibited by the UT-A inhibitor phloretin. CONCLUSIONS: Functional UT-A transporters are expressed in the plasma membranes of mouse colon, indicating that these proteins may play a key role in host/bacterial interaction.

Wolpert, E; Phillips, SF; Summerskill, WH. (1971) **Transport of urea and ammonia production in the human colon**. Lancet 2: 1387-1390.

4.5.2 Gene expression studies

Cohen, DM; Gullans, SR. (1993) **Urea induces Egr-1 and c-fos expression in renal epithelial cells**. Am J Physiol 264:F593–600. The membrane-permeant solute urea, in concentrations present in the mammalian renal medulla, increased expression at the mRNA level of two immediate-early gene (IEG) transcription factors, Egr-1 and c-fos, in a time- and dose-dependent fashion in confluent growth-

suppressed Madin-Darby canine kidney (MDCK) cells. This upregulation occurred in the absence of both cytotoxicity and an inhibition of protein synthesis, two potential nonspecific inducers of IEG expression. These findings were of interest because we have previously shown that hyperosmotic stress induced by the functionally membrane-impermeant solute NaCl increased expression of these IEG, whereas hyperosmotic stress induced by the membrane-permeant solute glycerol failed to do so. The urea-induced increase in Egr-1 mRNA expression was not secondary to enhanced message stability as determined by actinomycin D experiments and is therefore likely a consequence of urea-induced transcriptional activation. Augmented Egr-1 expression in response to urea treatment was also observed in another renal epithelial cell line, LLC-PK1, but not in other cell types examined. Therefore cells of renal epithelial origin may be uniquely capable of responding to hyperosmotic urea with increased expression of IEG transcription factors, and this increase is likely transcriptionally mediated.

Cohen, DM. (1999) **Signalling and gene regulation by urea and NaCl in the renal medulla**. Clin Exp Pharmacol Physiol 26: 69-73. 1. Cells of the mammalian renal medulla are routinely subjected to an enormously elevated and labile ambient osmolality as a consequence of the renal concentrating mechanism. The present review focuses on the most recent advances in hyperosmotic solute-mediated signal transduction and regulation of gene transcription in cells of the kidney medulla. 2. On the basis of osmolality alone, NaCl and urea are the principal renal medullary solutes. 3. Urea, which is membrane permeant, activates transcription of immediate-early genes via an extracellular signal-regulated kinase (ERK)/Elk-1-dependent pathway. Urea also activates multiple effectors characteristic of a receptor tyrosine kinase-like signalling cascade. 4. In contrast, the functionally impermeant solute NaCl activates transcription of tonicity responsive genes (principally genes encoding proteins essential for osmolyte uptake or synthesis) via a unique consensus element contained within their 5' flanking sequences. 5. An activity exhibiting tonicity inducible sequence-specific interaction with this DNA element has been identified. 6. Hypertonicity, like thermal stress, activates transcription of genes encoding heat shock proteins. The relationship between signalling events leading to tonicity mediated and heat shock-mediated gene transcription remains to be established.

Tian, W; Boss, GR; Cohen, DM. (2000) **Ras signaling in the inner medullary cell response to urea and NaCl**. Am J Physiol Cell Physiol 278: C372-380. The small guanine nucleotide-binding protein Ras, activated by peptide mitogens and other stimuli, regulates downstream signaling events to influence transcription. The role of Ras in solute signaling to gene regulation was investigated in the murine inner medullary collecting duct (mIMCD3) cell line. Urea treatment (100-200 mM), but not sham treatment, increased Ras activation 124% at 2 min; the effect of NaCl did not achieve statistical significance. To determine the contribution of Ras activation to urea-inducible signal transduction, mIMCD3 cells were stably transfected with an expression plasmid encoding a dominant negative-acting N17Ras mutant driven by a dexamethasone-inducible (murine mammary tumor virus) promoter. After 24 h of induction, selected cell lines exhibited sufficient N17Ras overexpression to abolish epidermal growth factor- and hypotonicity-mediated signaling to extracellular signal-regulated kinase (ERK) phosphorylation, as determined by immunoblotting. Conditional N17Ras overexpression inhibited urea- and NaCl-inducible ERK phosphorylation by 40-50%, but only at 15 min, and not 5 min, of treatment. N17Ras induction, however, almost completely inhibited urea-inducible Egr-1 transcription, as quantitated by luciferase reporter gene assay, but failed to influence tonicity-inducible (TonE-mediated) transcription. N17Ras overexpression also blocked urea-inducible expression of the transcription factor Gadd153 but did not influence osmotic or urea-inducible apoptosis. In addition, urea treatment induced recruitment of the Ras activator Sos to the plasma membrane. Taken together, these observations suggest a role for Ras signaling in the IMCD cell response to urea stress.

Tian, W; Cohen, DM. (2001) **Signaling and gene regulation by urea in cells of the mammalian kidney medulla**. Comp Biochem Physiol A Mol Integr Physiol 130: 429-436. Signaling by urea, although incompletely understood, is relevant both to cells of the mammalian kidney inner medulla and to all cells of the organism in the setting of advanced renal failure with its attendant accumulation of urea in the systemic circulation. The molecular events initiated by urea stress are distinct from those occurring in response to hypertonic stress; urea activates a characteristic subset of signaling events, which are in large part specific to cultured renal tubular epithelial cells. Interestingly, urea is protective of hypertonic NaCl-inducible apoptosis in this model. Details of this phenomenon are reviewed. The effect of urea has been likened to that of either hypertonicity or of a peptide mitogen. In preliminary expression array analyses, the profile of genes activated by urea stress in renal medullary cells, however, was found to be unique.

Tian, W; Cohen, DM. (2002) **Urea stress is more akin to EGF exposure than to hypertonic stress in renal medullary cells**. Am J Physiol Renal Physiol 283: F388-398. Although urea is considered to be a cell stressor even in renal medullary cells perpetually exposed to this solute in vivo by virtue of the renal concentrating mechanism, aspects of urea signaling resemble that of a peptide mitogen. Urea was compared with epidermal growth factor and hypertonic NaCl or hypertonic mannitol using a large-scale expression array-based approach. The expression profile in response to urea stress more closely resembled that of EGF treatment than hypertonic stress, as determined by hierarchical cluster analysis; the effect of urea+NaCl was equidistant from that of either solute applied individually. Among the most highly urea- and hypertonicity-responsive transcripts were genes that had previously been shown to be responsive to these solutes, validating this approach. Increased expression of the activating transcription factor 3 by urea was newly detected via expression array and confirmed via immunoblot analysis. Earlier, we noted an abrogation of tonicity-dependent gene regulation by urea, primarily in a transient transfection-based model (Tian W and Cohen DM. Am J Physiol Renal Physiol 280: F904-F912, 2001). Here we applied K-means cluster analysis to demonstrate that the genes most profoundly up- or downregulated by hypertonic stress were partially restored toward basal levels in the presence of urea pretreatment. These global expression data are consistent with our earlier biochemical studies suggesting that urea affords cytoprotection in this context. In the aggregate, these data strongly support the hypothesis that the urea effect in renal medullary cells resembles that of a peptide mitogen in terms of the adaptive program of gene expression and in terms of cytoprotection from hypertonicity.

Xu, H; Tian, W; Lindsley, JN; et al. (2005) **EphA2: expression in the renal medulla and regulation by hypertonicity and urea stress in vitro and in vivo**. Am J Physiol Renal Physiol 288: F855-866. EphA2, a member of the large family of Eph receptor tyrosine kinases, is highly expressed in epithelial tissue and has been implicated in cell-cell and cell-matrix interactions, as well as cell growth and survival. Expression of EphA2 mRNA and protein was markedly upregulated by both hypertonic stress and by elevated urea concentrations in cells derived from the murine inner medullary collecting duct. This upregulation likely required transactivation of the epidermal growth factor (EGF) receptor tyrosine kinase and metalloproteinase-dependent ectodomain cleavage of an EGF receptor ligand, based on pharmacological inhibitor studies. A human EphA2 promoter fragment spanning nucleotides -4030 to +21 relative to the putative EphA2 transcriptional start site was responsive to tonicity but insensitive to urea. A promoter fragment spanning -1890 to +128 recapitulated both tonicity- and urea-dependent upregulation of expression, consistent with transcriptional activation. Neither the bona fide p53 response element at approximately -1.5 kb nor a pair of putative TonE elements at approximately -3 kb conferred the tonicity responsiveness. EphA2 mRNA and protein were expressed at low levels in rat renal cortex but at high levels in the collecting ducts of the renal medulla and papilla. Water deprivation in rats increased EphA2 expression in renal papilla, whereas dietary supplementation with 20% urea increased EphA2 expression in outer medulla. These data indicate that transcription and expression of the EphA2

receptor tyrosine kinase are regulated by tonicity and urea in vitro and suggest that this phenomenon is also operative in vivo. Renal medullary EphA2 expression may represent an adaptive response to medullary hypertonicity or urea exposure.

Zhang, Z; Tian, W; Cohen, DM. (2000) **Urea protects from the proapoptotic effect of NaCl in renal medullary cells**. Am J Physiol Renal Physiol 279: F345-352. Hypertonic NaCl upregulated two sensitive and specific biochemical indices of apoptosis, caspase-3 activation and annexin V binding, in a time- and dose-dependent fashion in renal medullary mIMCD3 cells. Pretreatment with urea (200 mM for 30 min) protected from the proapoptotic effect of hypertonic stress (200 mosmol/kgH(2)O) in this model. The protective effect of urea was dose dependent and was effective even when applied a short time (< or =1 h) following NaCl exposure; this protective effect was not observed in the nonrenal 3T3 cell line. In both mIMCD3 and 3T3 cells, urea failed to protect from the proapoptotic stressor, ultraviolet (UV)-B irradiation. The ability of urea to protect from hypertonic stress was approximately comparable to the protective effect of peptide mitogens epidermal growth factor and insulin-like growth factor (IGF), but it potentiated the IGF effect. Interestingly, the tyrosine kinase inhibitor, genistein, potentiated the proapoptotic effect of urea yet abrogated the proapoptotic effect of hypertonic stress. In aggregate, these data indicate that urea protects from the proapoptotic effect of hypertonic stress in a potentially cell type-specific and stimulus-specific fashion.

Zhang, Z; Yang, X-Y; Cohen, DM. (1999) **Urea-associated oxidative stress and Gadd153/CHOP induction**. Am J Physiol 276: F786-93. Urea treatment (100-300 mM) increased expression of the oxidative stress-responsive transcription factor, Gadd153/CHOP, at the mRNA and protein levels (at ?4 h) in renal medullary mIMCD3 cells in culture, whereas other solutes did not. Expression of the related protein, CCAAT/enhancer-binding protein (C/EBP-?), was not affected, nor was expression of the sensor of endoplasmic reticulum stress, grp78. Urea modestly increased Gadd153 transcription by reporter gene anal. but failed to influence Gadd153 mRNA stability. Importantly, upregulation of Gadd153 mRNA and protein expression by urea was antioxidant sensitive. Accordingly, urea treatment was associated with oxidative stress, as quantitated by intracellular reduced glutathione content in mIMCD3 cells. In addition, antioxidant treatment partially inhibited the ability of urea to activate transcription of an Egr-1 luciferase reporter gene. Therefore, oxidative stress represents a novel solute-signaling pathway in the kidney medulla and, potentially, in other tissues.

Genotoxicity studies:

Chaurasia, OP; Sinha, SP. (1987) **Effects of urea on mitotic chromosomes of mice and onion**. Cytologia 52: 877-882.

Chaurasia, OP. (1991) **Randomness of chromosome breaks in bone marrow cells of fertilizer-fed mice, Mus musculus**. Cytobios 67: 7-12. Three commonly used fertilizers, urea, single superphosphate and muriate of potash, induced chromosome and chromatid breaks in the metaphase chromosomes of bone marrow cells of fertilizer-fed Swiss albino mice, Mus musculus. The breaks caused by urea and phosphate were non-randomly distributed, since they were more frequent in the longer chromosomes than in the smaller ones, and more common in the distal region than in the juxtacentromeric and median regions. The breaks induced by muriate of potash were randomly distributed in both the length and region of the chromosomes.

Cohen, DM; Gullans, SR. (1993) **Urea selectively induces DNA synthesis in renal epithelial cells**. Am J Physiol 264: F601-7. Hyperosmotic stress with the functionally impermeant solute NaCl has been shown by us and others to inhibit cell growth and DNA synthesis. Several lines of evidence suggest that urea, the other principal renal medullary solute, may exert a growth-promoting effect on renal epithelial cells. Among these is the finding that urea upregulates expression at the mRNA level of two growth-associated immediate-early genes, Egr-1 and c-fos. In the present study, urea, in concentrations characteristic of the renal medulla, increased [3H]thymidine incorporation approximately threefold in confluent, growth-suppressed Madin-Darby canine kidney (MDCK) cells, whereas another readily membrane-permeant solute, glycerol, did not. Urea also overcame the inhibitory effect of hyperosmotic NaCl on DNA synthesis. The urea-induced increase in [3H]thymidine incorporation was also evident in the renal epithelial LLC-PK1 cell line, but not in renal nonepithelial and epithelial nonrenal cell types examined. In addition, it was associated with a 15% increase in total DNA content measured fluorometrically at 24 h of treatment. There was, however, no associated increase in cell proliferation as measured by cell number, total protein content, or cell cycle distribution. Urea also failed to induce polyploidy or aneuploidy. Therefore cells of renal epithelial origin may be uniquely capable of responding to hyperosmotic urea with increased DNA synthesis through an undefined and potentially novel mechanism.

De Brabander, M; Van de Veire, R; Aerts, F; et al. (1976) **A new culture model facilitating rapid quantitative testing of mitotic spindle inhibition in mammalian cells**. J Natl Cancer Inst 56: 357-363. A new culture model, which facilitated both mass screening of potential anticancer drugs acting on microtubules and quantitative experiments with known "antitubulins," was found to have the following advantages: use of mammalian cells (either transformed or not), simplicity of the techniques (phase-contrast microscopy or simple microscopy after Giemsa staining), and ease with which it lent itself to quantification. The model was based on the uniform multimicronucleation response induced by antitubulins in MO cells. The specificity (towards antitubulins) of this response was ascertained by the use of many substances, including most of the known antitubulins and a number of nonrelated cytostatic or cytotoxic compounds. The uniformity of the response was established with the use of time-lapse observation of large numbers of cells and quantitative approaches. The results obtained in this model with the standard antitubulins (colchicine, vinblastine, vincristine) showed similar effects. The major difference between colchicine and the Vinca alkaloids was that colchicine was less reversible, which might be an indication of stronger intracellular binding. The Vinca alkaloids acted synergistically with colchicine when threshold subactive doses were combined, although it is known that they bind at a different site on tubulins. A number of substances that have been claimed or were suspected to interfere with microtubules were tested. The results showed that the following substances were indeed active with MO cells: colchicine, vinblastine, vincristine, podophyllotoxin, rotenone, griseofulvin, mercaptoethanol, benomyl, methyl benzimidazol-2-yl carbamate, and R 17934. Compounds that were inactive on these mammalian cells in culture included isopropyl carbanilate and melatonin, both of which have been shown to be active in other systems.

Fluck, ER; Poirier, LA; Ruelius, HW. (1976) **Evaluation of a DNA polymerase-deficient mutant of E. coli for the rapid detection of carcinogens**. Chem Biol Interact 15: 219-231. Differential growth inhibition of two E. coli cultures was evaluated as a rapid screening technique for chemical carcinogens. Of the carcinogens tested, only "direct acting" carcinogens produced positive results. Furthermore, this test is not a quantitative assay in that neither was a dose--response relationship seen nor did potent carcinogens necessarily show a greater response than weaker carcinogens.

Garberg, P; Akerblom, EL; Bolcsfoldi, G. (1988) **Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution**. Mutat

Res 203: 155-176. A rapid genotoxicity test, based on the measurement of the proportion of single- to double-stranded DNA by alkaline unwinding and hydroxyapatite elution in mouse lymphoma cells treated in vitro with various chemicals, was evaluated. Seventy-eight compounds from diverse chemical groups, including commonly tested mutagens, toxic compounds not usually tested for genotoxicity and non-toxic compounds not thought to be genotoxic were tested. The results obtained were compared with those from the mouse lymphoma TK locus forward-mutation assay, providing a basis for assessing the relative sensitivity of the 2 assays using the same cells exposed to chemicals under similar conditions. Clear evidence of DNA-damaging activity was obtained with 43 of the compounds, while 4 gave equivocal results. Of the remaining 31 compounds, 14 were toxic without inducing DNA damage while the rest were non-toxic and did not induce any DNA damage. Results were available from both the alkaline unwinding assay and the mouse lymphoma assay for 61 compounds; they showed a concordance between the 2 assays of 77%. Of the 47 compounds that were positive or equivocal in the alkaline unwinding assay, only carbon tetrachloride and prednisolone were negative in the mouse lymphoma assay, while 12 of the 19 compounds that were negative in the alkaline unwinding assay were positive in the mouse lymphoma assay. These included 3 compounds that interfere with nucleic acid metabolism, and 3 crosslinking agents, which would be expected to produce mutations to a greater extent than strand breaks. The other 6 compounds were anthranilic acid, benzoquinone, p-chloroaniline, diethylmaleate, glucose and procarbazine HCl. Of these only the last is a known carcinogen. It is concluded from the present study that there was good overall agreement between the results of the DNA alkaline unwinding and mouse lymphoma TK locus assays, but that the sensitivity of the alkaline unwinding assay is lower for some classes of compounds. Bearing this in mind, the alkaline unwinding assay is considered suitable as a rapid screen for genotoxic activity in eukaryotic cells.

Hellmer, L; Bolcsfoldi, G. (1992) **An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds**. Mutat Res 272: 145-160. A differential DNA repair test was evaluated in vitro, using derivatives of E. coli K-12 343/113 with the genotype uvrB-/recA- and uvrB+/recA+. The aim of this study was to characterize the sensitivity of the assay to different compounds in vitro and thereby provide information on the usefulness of this end-point as an indicator of genotoxicity in a host-mediated assay. Sixty-one compounds from diverse chemical groups were tested and of these 32 gave a positive result. The results obtained were compared with results from the Ames test and were in agreement for 49 out of the 61 compounds tested. Chemicals that were detected in this test but negative in the Ames test were 4-aminophenol, catechol, diethylstilbestrol, thioacetamide and thiourea. Seven of the compounds tested gave a negative result in E. coli but were positive in Salmonella. These were 4-aminobiphenyl, benzo[a]pyrene, cyclophosphamide, 1-naphthylamine, N-nitrosobutylpropylamine, quinoline and 2-toluidine. The performance of the in vitro test and reasons for the discrepant results with the Ames test are discussed. The overall concordance between the two tests was about 80%. On the basis of these results we consider these bacterial strains, and differential DNA repair as an end-point, to be sufficiently accurate as an indicator of genotoxicity in vitro and thereby also in vivo.

Ishidate, M Jr.; Odashima, S. (1977) **Chromosome tests with 134 compounds on Chinese hamster cells in vitro--a screening for chemical carcinogens**. Mutat Res 48: 337-353. Chromosomal aberration tests in vitro were carried out on Chinese hamster cells grown in culture with various chemicals, including carcinogenic N-nitroso compounds and their related derivatives, food additives, medical drugs, pesticides and other chemicals commonly used in laboratories or industries. Sixty-three of the 134 chemicals gave negative results in our test system even with doses at which the cell growth was markedly inhibited. Nearly all compounds known to be mutagenic in bacteria were also positive in our tests. Both urethane and diethylstilbestrol were positive, even though they are known to be carcinogenic but not mutagenic in bacteria. Compounds such as N-alkyl-N'-nitroguanidines, barbital, sodium benzoate,

saccharin sodium, sodium nitrite, sodium nitrate and 4-aminoquinoline-1-oxide were positive in our chromosome tests, but they have not been conclusively tested for their carcinogenicity.

Ishidate, M Jr.; Yoshikawa, K. (1980) **Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation--a comparative study on mutagens and carcinogens**. Arch Toxicol Suppl 4: 41-44. Chromosome aberration tests (CH-test) in vitro were carried out on more than 400 chemicals from our environment, which included carcinogens and other compounds such as food additives, medical drugs, pesticides and those used in laboratories or industries. All results were compared with those obtained by mutation assays with bacteria (Ames test). Nearly half of these chemicals tested were positive either in the CH-tests or in the Ames tests. Among the chemicals being positive in the CH-tests, however, there were some which were negative in the Ames tests but have been proved to be carcinogenic in animals. Mammalian cell systems, therefore, may be not dispensable and are postulated for the primary screening for chemical mutagens and carcinogens in our environment.

Kommadath, A; Sharma, A; Jakhar, KK. (2001) **Hepatotoxic, nephrotoxic and genotoxic effects in mice fed urea adulterated milk**. Indian J Dairy Sci 54: 316-321.

Kultz, D; Chakravarty, D. (2001) **Hyperosmolality in the form of elevated NaCl but not urea causes DNA damage in murine kidney cells**. Proc Natl Acad Sci USA 98: 1999-2004.

Matthews, EJ; Spalding, JW; Tennant, RW. (1993) **Transformation of BALB/c-3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in Salmonella and carcinogenicity in rodent bioassays**. Environ Health Perspect 101 Suppl 2: 347-482. This report describes the activities of 168 chemicals tested in a standard transformation assay using A-31-1-13 BALB/c-3T3 cells. The data set includes 84 carcinogens, 77 noncarcinogens, and 7 research chemicals. Carcinogens included 49 mutagens and 35 nonmutagens; noncarcinogens included 24 mutagens and 53 nonmutagens. The transformation assay did not use an exogenous activation system, thus, all chemical responses depended on the inherent target cell metabolic capacity where metabolic activation was required. The upper dose limit was 100 milli-osmolar because the assay could not discriminate active and inactive chemicals tested above this concentration. Certain physicochemical properties resulted in technical problems that affected chemical biological activity. For example, chemicals that reacted with plastic were usually nonmutagenic carcinogens. Similarly, chemicals that were insoluble in medium, or bound metals, were usually nonmutagenic and nontransforming. Multifactorial data analyses revealed that the transformation assay discriminated between nonmutagenic carcinogens and noncarcinogens; it detected 64% of the carcinogens and only 26% of the noncarcinogens. In contrast, the transformation assay detected most mutagenic chemicals, including 94% of the mutagenic carcinogens and 70% of the mutagenic noncarcinogens. Thus, transformation or Salmonella typuimurium mutagenicity assays could not discriminate mutagenic carcinogens from mutagenic noncarcinogens. Data analyses also revealed that mutagenic chemicals were more cytotoxic than nonmutagenic chemicals; 88% of the mutagens had an LD50 < 5 mM, whereas half of the nonmutagens had an LD50 > 5 mM. Binary data analyses of the same data set revealed that the transformation assay and rodent bioassay had a concordance of 71%, a sensitivity for carcinogens of 80.0%, and a specificity for detecting noncarcinogens of 60%. In contrast, Salmonella mutagenicity assays and rodent bioassays had a concordance of 63%, a sensitivity of 58%, and a specificity of 69%. The transformation assay complemented the Salmonella mutagenesis assay in the identification of nonmutagenic carcinogens; thus, the two assays had a combined 83% sensitivity for all carcinogens and a 75% specificity for nonmutagenic noncarcinogens.

Mortelmans, K; Haworth, S; Lawlor, T; et al. (1986) **Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals**. Environ Mutagen 8 Suppl 7: 1-119. This publication includes data of

Salmonella mutagenicity results on 270 coded chemicals, encompassing 329 tests performed by three laboratories under contract to the National Toxicology Program (NTP). The preincubation modification of the Salmonella/mammalian microsome assay was used to test chemicals in up to five Salmonella strains in the presence and absence of rat and hamster liver S-9. With a few exceptions, inter- and intralaboratory reproducibility was good.

Nesslany, F; Marzin, D. (1999) **A micromethod for the in vitro micronucleus assay**. Mutagenesis 14: 403-410. A micromethod for the in vitro micronucleus assay was developed using L5178Y cells to enable the rapid screening of a large number of molecules. The method is quick, simple to perform and needs very small amounts of compound, i.e. <10 mg. In this methodology, three types of treatment were carried out in parallel, enabling an optimal detection of both aneugenic and clastogenic compounds: two treatments without metabolic activation with or without a recovery period after a 24 h continuous treatment and one treatment with metabolic activation by Aroclor 1254-induced rat or hamster liver S9 mix. Seventeen known genotoxins (12 clastogens and five aneugens) and seven known non-genotoxins were tested. The in vitro micronucleus micromethod using L5178Y cells exhibited good sensitivity (16 positive/17 known genotoxins tested) and specificity (7 negative/7 known non-genotoxins tested) for the 24 test compounds studied with or without metabolic activation. Furthermore, this test showed a good correlation with other in vitro micronucleus tests performed using macromethods with various mammalian cell cultures. We conclude that the in vitro micronucleus micromethod with L5178Y cells could be used in the earliest stages of development of new molecules as a preliminary short-term screening assay before starting regulatory tests.

Oppenheim, JJ; Fishbein, WN. (1965) **Induction of chromosome breaks in cultured normal human leukocytes by potassium arsenite, hydroxyurea and related compounds**. Cancer Res 25: 980-985.

Shimizu, H; Suzuki, Y; Takemura, N; et al. (1985) **The results of microbial mutation test for forty-three industrial chemicals**. Sangyo Igaku 27: 400-419. The mutagenicity of 43 industrial chemicals in Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538) and Escherichia coli (WP2uvrA) was examined. The output of these chemicals in Japan is more than a million kilograms per year. The mutation test was carried out under the condition of absence and presence of rat microsomal activation. Two chemicals, hexamethylenetetramine and 4,4'-methylenediphenyldiisocyanate, showed mutagenic activity in S. typhimurium TA98 and TA100 by metabolic activation. Hexamethylenetetramine also showed mutagenic activity in TA98 without microsomal activation. No mutagenic activity was observed in the 41 chemicals including 4 volatile and gaseous compounds.

Sina, JF; Bean, CL; Dysart, GR; et al. (1983) **Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential**. Mutat Res 113: 357-391. An alkaline elution/rat hepatocyte assay was developed to sensitively measure DNA single-strand breaks induced by xenobiotics in nonradiolabeled rat hepatocytes. This assay was evaluated as a predictor of carcinogenic/mutagenic activity of 91 compds. (64 carcinogens and 27 noncarcinogens) from >25 diverse chemical classes. Hepatocytes were isolated from uninduced rats by collagenase perfusion, exposed to chems. for 3 h, harvested, and analyzed for DNA single-strand breaks by alkaline elution. DNA detns. were done fluorimetrically. Cytotoxicity was estimated by glutamate-oxaloacetate transaminase release or by trypan blue dye exclusion. The assay correctly predicted the reported carcinogenic/noncarcinogenic potential of 92% of the carcinogens tested and 85% of the noncarcinogens tested. The assay detected a number of compds., including inorgs., certain pesticides, and steroids, which give false-neg. results in other short-term tests. Only 2 rat liver carcinogens were incorrectly identified; the other carcinogens incorrectly identified are weakly or questionably carcinogenic (i.e., they cause tumors only in 1 species, after lifetime exposure, or at high doses). Some chems. cause DNA damage only at cytotoxic concns.; of 16

such compds. in this study, 12 are weak carcinogens suggesting a link between DNA damage caused by cytotoxicity and carcinogenesis. This assay rapidly, reproducibly, sensitively, and accurately detects DNA single-strand breaks in rat hepatocytes and the production of these breaks correlates well with carcinogenic and mutagenic activity.

Szybalski, W. (1958). **Special microbiological systems. 2. Obsevations on chemical mutagenesis in microorganisms**. Ann N Y Acad Sci 76: 475-489.

Wangenheim, J; Bolcsfoldi, G. (1986). **Mouse lymphoma TK+/- assay of 30 compounds**. Environ Mutagen 8: 90.

Wangenheim, J; Bolcsfoldi, G. (1988) **Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds**. Mutagenesis 3: 193-205. Mutagenicity results are presented for 50 compounds tested in the mouse lymphoma TK+/(-)----TK-/- forward mutation assay. Test compounds were mostly from chemical classes not previously tested, to provide new information on the sensitivity of the assay; chemicals of low toxicity or thought to be non-carcinogenic and metabolic inhibitors, to indicate whether and under what conditions the assay can generate so-called false positive results. Twelve compounds that have been tested previously were included in this study to provide an indication of the reproducibility of the assay. Concordant results were obtained for nine of these, while disagreeing, positive results were seen with aniline, fluorene and pyrene. The following compounds belonging to the noncarcinogen category were positive at concentrations in the range 0.02-1 mol/l: dimethyl sulphoxide, EDTA, glucose, polyethyleneglycol, sodium chloride, sodium nitrite and urea. Measurements of the osmotic pressure indicated a lack of a simple relationship to mutagenic effects for these compounds. While the potent mutagenic/carcinogenic compounds tested gave greater than 4-fold increases in the mutation frequency, weak carcinogens or compounds not known to be carcinogenic that were positive in the assay gave increases of between 2- and 4-fold. Exceptions were aldehyde derivatives and chemicals that can lead to oxidative stress, which were detected with exaggerated sensitivity by the assay. Among the metabolic inhibitors tested, positive results were obtained with actinomycin D, cycloheximide, diethyl maleate, hydroxyurea and ouabain. Negative results were found with antimycin A. On the basis of the present results and previously published data it is concluded that a maximum limit for the test compound concentration can be set at 20 mmol/l and that testing to 20% total growth is adequate, with certain stipulations, to detect the mutagenic activity of test compounds. A similar analysis of the available test data shows that less than 4-fold increases in the mutation frequency have a lower predictivity for carcinogenicity.

Topham, JC. (1980) **Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens?** Mutat Res 74: 379-387.

Tuite, MF; Mundy, CR; Cox, BS. (1981) **Agents that cause a high frequency of genetic change from [psi+] to [psi-] in Saccharomyces cerevisiae**. Genetics 98: 691-711. The [psi] factor of yeast is cytoplasmically inherited. Singh, Helms and Sherman (1979) reported that high concentrations of KCl and of ethylene glycol induce the genetic change from [psi+] to [psi-]. In this study, the following agents have been shown to induce the same genetic change: guanidine hydrochloride at 1 mM, dimethyl sulfoxide at 2.5% v/v and ethanol or methanol at 10% v/v. It is likely that a number of other agents also cause the change, namely 2 M glycerol, M succinate, M glutamate and M MgCl2. Most of these agents induce the change at very high frequencies; with some, the frequency is 100%. Although the observed phenotypic change can also occur as a result of chromosomal gene mutation, no changes of this type were identified. Some of the agents also cause mutation from [rho+] to [rho-] and from killer to sensitive.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

See also information in Sections 2 and 4.5.

Bagnasco, SM. (2005) **Role and regulation of urea transporters**. Pflugers Arch 450: 217-226. In the past few years, significant knowledge has been gained about the physiological role and regulation of urea transporters, which have been now cloned in many species. The two major mammalian urea transporters, UT-A and UT-B, have been best studied in the kidney, where they mediate the facilitated diffusion of urea across tubular, interstitial, and vascular compartments, necessary to maintain an osmolar gradient along the renal corticomedullary axis. The genes encoding these transporters, Slc14A2 for UT-A and Slc14A1 for UT-B, have been characterized in rodents and humans, allowing identification of transcriptional mechanisms involved in the regulation of UT-A expression. The crucial role that urea transporters play in renal physiology is underscored by the phenotypic characteristics of UT-A and UT-B knockout mice, in which lack of specific urea transporters impairs the ability to concentrate urine. Expression of the UT-A and UT-B transporters has also been identified in extra-renal sites, where their physiological significance is only beginning to be elucidated. More information on the mechanisms modulating urea transporter expression is becoming available, and the possible involvement of aberrant regulation of these transporters in pathological conditions, or as a result of certain pharmacological treatments, has emerged from recent studies.

Bagnasco, SM. (2006) **The erythrocyte urea transporter UT-B**. J Membr Biol 212: 133-138. During the past decade significant progress has been made in our understanding of the role played by urea transporters in the production of concentrated urine by the kidney. Urea transporters have been cloned and characterized in a wide range of species. The genomic organization of the two major families of mammalian urea transporters, UT-A and UT-B, has been defined, providing new insight into the mechanisms that regulate their expression and function in physiological and pathological conditions. Beside the kidney, the presence of urea transporters has been documented in a variety of tissues, where their role is not fully known. Recently, mice with targeted deletion of the major urea transporters have been generated, which have shown variable impairment of urine concentrating ability, and have helped to clarify the physiological contribution of individual transporters to this process. This review focuses on the erythrocyte urea transporter UT-B.

Bankir, LT; Trinh-Trang-Tan, MM. (2000) **Renal urea transporters. Direct and indirect regulation by vasopressin**. Exp Physiol 85 Spec No: 243S-252S. Urea is the most abundant urinary solute and is excreted in urine at a much higher concentration than in other body fluids. Urea concentration is achieved in the kidney through complex urea movements between blood vessels and renal tubules, which involve facilitated urea transport. Three major urea transporters expressed in the kidney have been cloned, UT-A1, UT-A2 and UT-B1, the first two derived from the same gene by differential transcription. These membrane proteins enable facilitated diffusion of urea through specific parts of the nephron (UT-A) and through renal vasculature (UT-B) in the medulla. UT-A1 is localised in the terminal part of the inner medullary collecting ducts and accounts for the vasopressin-dependent increase in urea permeability of this segment. UT-A2 is found in the descending thin limbs of Henle's loops. UT-B1 is expressed in the endothelium of the descending vasa recta supplying blood to the renal medulla, and in red cells. All three urea transporters are primarily involved in the process of intrarenal urea recycling, which enables the establishment, and prevents the dissipation, of a high concentration of urea in the inner medulla. This is an essential feature for producing a concentrated urine and thus for water economy in mammals. Vasopressin, upon binding to V2 receptors in the inner medullary collecting ducts, increases urea

permeability through activation of UT-A1 molecules, thus enabling urea to diffuse into the inner medullary interstitium. Urea then taken up in ascending vasa recta is returned to the inner medulla via UT-A2 and UT-B1 by countercurrent exchange. These latter two urea transporters are not influenced acutely by vasopressin, but UT-A2 expression is markedly increased in the descending thin limbs of the loops of Henle after sustained exposure to vasopressin or its V2 agonist dDAVP. This effect is indirect because vasopressin receptors are lacking in the descending limbs. The acute direct and delayed indirect actions of vasopressin on renal urea transporters will increase medullary urea accumulation and thus the ability of the kidney to conserve water. Atrial natriuretic peptide inhibits the vasopressin-dependent increase in urea permeability in the inner medullary collecting ducts. The interruption of urea recycling probably contributes to the natriuresis. Impairing in this way the capacity of the kidney to concentrate urea enhances its capacity to concentrate sodium in the urine.

Kraus, LM; Kraus, AP Jr. (1998) **The search for the uremic toxin. The case for carbamoylation of amino acids and proteins**. Wien Klin Wochenschr 110: 521-530. A review with 97 refs. is given on the role cyanate as uremic toxin. The following topics are included: cyanate in sickle-cell anemia and uremia, carbamoylation of amino acids, Hb, proteins, enzymes, hormones, antibodies, and low-d. lipoprotein, carbamoylation and glycosylation, changed structure and altered function by carbamoylation, the uremic status assessed by homocitrulline, and carbamoylation in normal subjects.

Sands, JM. (1999) **Regulation of renal urea transporters**. J Am Soc Nephrol 10: 635-646. Urea is important for the conservation of body water due to its role in the production of concentrated urine in the renal inner medulla. Physiologic data demonstrate that urea is transported by facilitated and by active urea transporter proteins. The facilitated urea transporter (UT-A) in the terminal inner medullary collecting duct (IMCD) permits very high rates of transepithelial urea transport and results in the delivery of large amounts of urea into the deepest portions of the inner medulla where it is needed to maintain a high interstitial osmolality for concentrating the urine maximally. Four isoforms of the UT-A urea transporter family have been cloned to date. The facilitated urea transporter (UT-B) in erythrocytes permits these cells to lose urea rapidly as they traverse the ascending vasa recta, thereby preventing loss of urea from the medulla and decreasing urine-concentrating ability by decreasing the efficiency of countercurrent exchange, as occurs in Jk null individuals (who lack Kidd antigen). In addition to these facilitated urea transporters, three sodium-dependent, secondary active urea transport mechanisms have been characterized functionally in IMCD subsegments: (1) active urea reabsorption in the apical membrane of initial IMCD from low-protein fed or hypercalcemic rats; (2) active urea reabsorption in the basolateral membrane of initial IMCD from furosemide-treated rats; and (3) active urea secretion in the apical membrane of terminal IMCD from untreated rats. This review focuses on the physiologic, biophysical, and molecular evidence for facilitated and active urea transporters, and integrative studies of their acute and long-term regulation in rats with reduced urine-concentrating ability.

Sands, JM. (2000) **Regulation of urea transporter proteins in kidney and liver**. Mt Sinai J Med 67: 112-119. Due to urea's role in producing concentrated urine, its transport is critically important to the conservation of body water. Within the renal inner medulla, urea is transported by both facilitated and active urea transport mechanisms. The vasopressin-regulated, facilitated urea transporter (UT-A1) in the terminal inner medullary collecting duct (IMCD) permits high rates of transepithelial urea transport and results in delivery of large quantities of urea into the deepest portions of the inner medulla where it is needed to maintain a high interstitial osmolality for maximal urine concentration. Four cDNA isoforms of the UT-A urea transporter family have been cloned. In addition, there are three secondary active, sodium-dependent, urea transport mechanisms in IMCD subsegments: (1) active urea secretion in the apical membrane of the terminal IMCD from untreated rats; (2) active urea absorption in the apical membrane of the initial IMCD from low-protein fed or hypercalcemic rats; and (3) active urea absorption in the

basolateral membrane of the initial IMCD from furosemide-treated rats. This review will focus on integrative studies of the rapid and long-term regulation of urea transporters in rats with reduced urine concentrating ability. These studies led to the surprising result that the basal-facilitated urea permeability in the terminal IMCD and UT-A1 protein abundance are increased during in vivo conditions associated with an impaired urine concentrating ability. In contrast, there are two response patterns of active urea transporters: (1) hypercalcemia, a low-protein diet, and furosemide result in induction of active urea absorption in the initial IMCD, albeit by different mechanisms, and inhibition of active urea secretion in the terminal IMCD; while (2) water diuresis results in up-regulation of active urea secretion in the terminal IMCD without any active urea absorption in the initial IMCD. The first pattern contributes to the urine concentrating defect by increasing urea delivery to the base of the inner medulla, thus decreasing urea delivery distally to the inner medullary tip. The second response pattern will directly decrease urea content in the deep inner medulla. UT-A urea transporters are also expressed outside the kidney. Recent studies show that the liver has phloretin-inhibitable urea transport and that it occurs via a 49 kDa UT-A protein. When rats are made uremic, the abundance of this 49 kDa UT-A protein increases in the liver in vivo. This up-regulation of the 49 kDa UT-A protein may allow hepatocytes to increase ureagenesis to reduce the accumulation of ammonium and/or bicarbonate in uremia.

Sands, JM. (2003) **Molecular mechanisms of urea transport**. J Membr Biol 191: 149-163. Physiologic data provided evidence for specific urea transporter proteins in red blood cells and kidney inner medulla. During the past decade, molecular approaches resulted in the cloning of several urea transporter cDNA isoforms derived from two gene families: UT-A and UT-B. Polyclonal antibodies were generated to the cloned urea transporter proteins, and their use in integrative animal studies resulted in several novel findings, including: (1) UT-B is the Kidd blood group antigen; (2) UT-B is also expressed in many non-renal tissues and endothelial cells; (3) vasopressin increases UT-A1 phosphorylation in rat inner medullary collecting duct; (4) the surprising finding that UT-A1 protein abundance and urea transport are increased in the inner medulla during conditions in which urine concentrating ability is reduced; and (5) UT-A protein abundance is increased in uremia in both liver and heart. This review will summarize the knowledge gained from studying molecular mechanisms of urea transport and from integrative studies into urea transporter protein regulation.

Sands, JM. (2003) **Mammalian urea transporters**. Annu Rev Physiol 65: 543-566. Urea plays a key role in the urine-concentrating mechanism. Physiologic and molecular data demonstrate that urea transport in kidney and red blood cells occurs by specific urea transporter proteins. Two gene families for facilitated urea transporters, UT-A and UT-B, and several urea transporter cDNA isoforms have been cloned from human, rat, mouse, and several non-mammalian species. Polyclonal antibodies have been generated to many of the urea transporter proteins, and several novel findings have resulted from their use in integrative animal studies. For example, (a) vasopressin increases the phosphorylation of UT-A1 in rat inner medullary collecting duct; (b) UT-A1 protein abundance is increased in the rat inner medulla during conditions in which urine-concentrating ability is reduced; and (c) urea transporters are expressed in non-renal tissues, and UT-A protein abundance is up-regulated in uremia in both liver and heart. In addition to the facilitated urea transporters, functional evidence exists for active urea transport in the kidney collecting duct. This review summarizes the physiologic evidence for the existence of facilitated and active urea transporters, the molecular biology of the facilitated urea transporter gene families and cDNAs, and integrative studies into urea transporter protein regulation, both in the kidney and in other organs.

Vanholder, R. (2003) **Uremic toxins**. Nephrologie 24: 373-376. A review. Uremic toxins are compds. which exert biol. action and which are retained in the body of patients with renal failure, whereas they normally should be excreted by the healthy kidneys into the urine. Only few retention solutes conform

with the strict definition of uremic toxins. Uremic solutes can be subdivided into small water-soluble mols., small protein-bound mols. and middle mols. Most small water-soluble compds. are not very toxic and the toxic ones often show a kinetic behavior that is different from that of urea. The uremic solutes that play a role in inflammation and cardiovascular complications most often are middle mols. and/or protein bound. The current marker urea is not representative for their mechanistic behavior.

4.7. EVALUATION OF CARCINOGENICITY

See also information in Sections 2 and 4.5.

Kirkland, D; Aardema, M; Henderson, L; et al. (2005) **Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity. [Erratum to document cited in CA143:243161]**. Mutat Res 588: 70. On the title page, the URL of the website address in the open star footnote should read: www.lhasalimited.org/cgx. This is where the appendixes have been posted.

Kirkland, DJ; Henderson, L; Marzin, D; et al. (2005) **Testing strategies in mutagenicity and genetic toxicology: an appraisal of the guidelines of the European Scientific Committee for Cosmetics and Non-Food Products for the evaluation of hair dyes**. Mutat Res 588: 88-105. The European Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) guideline for testing of hair dyes for genotoxic/mutagenic/carcinogenic potential has been reviewed. The battery of six in vitro tests recommended therein differs substantially from the batteries of two or three in vitro tests recommended in other guidelines. Our evaluation of the chemical types used in hair dyes and comparison with other guidelines for testing a wide range of chemical substances, lead to the conclusion that potential genotoxic activity may effectively be determined by the application of a limited number of well-validated test systems that are capable of detecting induced gene mutations and structural and numerical chromosomal changes. We conclude that highly effective screening for genotoxicity of hair dyes can be achieved by the use of three assays, namely the bacterial gene mutation assay, the mammalian cell gene mutation assay (mouse lymphoma tk assay preferred) and the in vitro micronucleus assay. These need to be combined with metabolic activation systems optimised for the individual chemical types. Recent published evidence [D. Kirkland, M. Aardema, L. Henderson, L. Muller, Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1-256] suggests that our recommended three tests will detect all known genotoxic carcinogens, and that increasing the number of in vitro assays further would merely reduce specificity (increase false positives). Of course there may be occasions when standard tests need to be modified to take account of special situations such as a specific pathway of biotransformation, but this should be considered as part of routine testing. It is clear that individual dyes and any other novel ingredients should be tested in this three-test battery. However, new products are formed on the scalp by reaction between the chemicals present in hair-dye formulations. Ideally, these should also be tested for genotoxicity, but at present such experiences are very limited. There is also the possibility that one component could mask the genotoxicity of another (e.g. by being more toxic), and so it is not practical at this time to recommend routine testing of complete hair-dye formulations as well. The most sensible approach would be to establish whether any reaction products within the hair-dye formulation penetrate the skin under normal conditions of use and test only those that penetrate at toxicologically relevant levels in the three-test in vitro battery. Recently published data [D. Kirkland, M. Aardema, L. Henderson, L. Muller, Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and

relative predictivity, Mutat. Res. 584 (2005) 1-256] suggest the three-test battery will produce a significant number of false as well as real positives. Whilst we are aware of the desire to reduce animal experiments, determining the relevance of positive results in any of the three recommended in vitro assays will most likely have to be determined by use of in vivo assays. The bone marrow micronucleus test using routes of administration such as oral or intraperitoneal may be used where the objective is extended hazard identification. If negative results are obtained in this test, then a second in vivo test should be conducted. This could be an in vivo UDS in rat liver or a Comet assay in a relevant tissue. However, for hazard characterisation, tests using topical application with measurement of genotoxicity in the skin would be more appropriate. Such specific site-of-contact in vivo tests would minimise animal toxicity burden and invasiveness, and, especially for hair dyes, be more relevant to human routes of exposure, but there are not sufficient scientific data available to allow recommendations to be made. The generation of such data is encouraged.

Kirkland, D; Aardema, M; Muller, L; et al. (2006) **Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens II. Further analysis of mammalian cell results, relative predictivity and tumour profiles**. Mutat Res 608: 29-42. One of the consequences of the low specificity of the in vitro mammalian cell genotoxicity assays reported in our previous paper [D. Kirkland, M. Aardema, L. Henderson, L. Muller, Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1-256] is industry and regulatory agencies dealing with a large number of false-positive results during the safety assessment of new chemicals and drugs. Addressing positive results from in vitro genotoxicity assays to determine which are "false" requires extensive resources, including the conduct of additional animal studies. In order to reduce animal usage, and to conserve industry and regulatory agency resources, we thought it was important to raise the question as to whether the protocol requirements for a valid in vitro assay or the criteria for a positive result could be changed in order to increase specificity without a significant loss in sensitivity of these tests. We therefore analysed some results of the mouse lymphoma assay (MLA) and the chromosomal aberration (CA) test obtained for rodent carcinogens and non-carcinogens in more detail. For a number of chemicals that are positive only in either of these mammalian cell tests (i.e. negative in the Ames test) there was no correlation between rodent carcinogenicity and level of toxicity (we could not analyse this for the CA test as insufficient data were available in publications), magnitude of response or lowest effective positive concentration. On the basis of very limited in vitro and in vivo data, we could also find no correlation between the above parameters and formation of DNA adducts. Therefore, a change to the current criteria for required level of toxicity in the MLA, to limit positive calls to certain magnitudes of response, or to certain concentration ranges would not improve the specificity of the tests without significantly reducing the sensitivity. We also investigated a possible correlation between tumour profile (trans-species, trans-sex and multi-site versus single-species, single-sex and single-site) and pattern of genotoxicity results. Carcinogens showing the combination of trans-species, trans-sex and multi-site tumour profile were much more prevalent (70% more) in the group of chemicals giving positive results in all three in vitro assays than amongst those giving all negative results. However, single-species, single-sex, single-site carcinogens were not very prevalent even amongst those chemicals giving three negative results in vitro. Surprisingly, when mixed positive and negative results were compared, multi-site carcinogens were highly prevalent amongst chemicals giving only a single positive result in the battery of three in vitro tests. Finally we extended our relative predictivity (RP) calculations to combinations of positive and negative results in the genotoxicity battery. For two out of three tests positive, the RP for carcinogenicity was no higher than 1.0 and for 2/3 tests negative the RP for non-carcinogenicity was either zero (for Ames+MLA+MN) or 1.7 (for Ames+MLA+CA). Thus, all values were less than a meaningful RP of two, and indicate that it is not possible to predict outcome of the rodent carcinogenicity study when only 2/3 genotoxicity results are in agreement.

Visek, WJ; Zimber, A. (1971) **Urea hydrolysis and colon cancer a parallelism**. Fed Proc 30: 565.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

No studies on susceptible populations were found.