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Final Report of the Safety Assessment of Urea[1]

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 ($\times 10^6$) and 1.20 ± 0.09 ($\times 10^3$) cm/min, respectively. The absorption of Urea across normal and abraded human skin was $9.5\% \pm 2.3\%$ and $67.9\% \pm 5.6\%$, 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.

CHEMISTRY

Definition and Structure

Urea (CAS no. 57-13-6) is an amide and conforms to the formula in Figure 1.

Synonyms for Urea include Carbamide (Wenninger et al. 2000; Registry of Toxic Effects of Chemical Substances [RTECS] 1999); Carbamide resin; Carbamimidic acid; Carbonyl diamide; Carbonyldiamine; Isourea; 75 Urea; Pseudourea; Ureaphil; Ureophil; Urevert (RTECS 1999).

Chemical and Physical Properties

The chemical and physical properties for Urea are given in Table 1.

Lee et al. (1995) stated that the hydrogen-bonded, cyclic Urea dimer is the most stable conformation with a binding energy of −10.9 kcal/mol. However, due to the hydrogen bonding between Urea and water, the dimer would become unstable and separate

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[1]Reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. This report was prepared by Torill A. Yamarik and Amy R. Elmore, former CIR staff.

TABLE 1
Chemical and physical properties of and specifications for urea

Property	Description	Reference
Appearance/odor	Colorless to white, prismatic crystals, or white crystalline powder, or small white pellets; odorless, but may develop a slight odor of ammonia upon standing	Committee of Revision of the United States Pharmacopeial Convention 1995; Gennaro 1990; National Academy of Sciences (NAS) 1996
	Saline taste	Gennaro 1990; Lewis 1993a
Boiling point	Decomposes	Lewis 1993a, 1993b
Freezing point	132.7°C	Wilkinson and Moore 1982; Lewis 2000
Density	1.335 (solid)	Lewis 1993b, 2000
Solubility	Solutions are neutral in litmus; freely soluble in water and boiling alcohol, practically insoluble in chloroform and in ether	Committee of Revision of the United States Pharmacopeial Convention 1995; NAS 1996
	Soluble in water, alcohol and benzene, slightly soluble in ether, almost insoluble in chloroform	Lewis 1993a
	In lipid = −1.563 mol/L	Chessells et al. 1992
	In water = 1000 g/L; octanol = 2.0 g/L	Bronaugh and Stewart 1985
Molecular weight	60.06	Gennaro 1990
Octanol/water partition coefficient	−2.11	Hui et al. 1995; Bronaugh and Stewart 1985
Powdered human stratum corneum/water partition coefficient	At concentrations of 0.03%, 0.06%, and 0.12% (*w/v*) the mean partition coefficient was 0.26 ± 0.02, 0.15 ± 0.02, and 0.17 ± 0.02, respectively	Hui et al. 1995
Ether/water partition coefficient	−3.49	Ackermann and Flynn 1987
Ether/water distribution	4.7×10^{-4}	Rapoport et al. 1972
Olive oil/water distribution	1.6×10^{-4}	Rapoport et al. 1972
pKa	0.18	Hui et al. 1995
pH in water	2.1	Hui et al. 1995
Ionization	Partially ionized in water or ionized in water	Hui et al. 1995
Melting point	135°C	Grasselli 1973
	132–134°C	Nikitakis and McEwen 1990b
	132–135°C	United States Pharmacopeial Convention, Inc. 2000
	Specifications	
Purity	99.5% minimum (cosmetic grade)	Nikitakis and McEwen 1990a
Impurities		
Chloride (as Cl)	70 ppm maximum	Nikitakis and McEwen 1990a
Sulfate (as SO_4)	100 ppm maximum	Nikitakis and McEwen 1990a
Sulfated Ash	0.05% maximum	Nikitakis and McEwen 1990a
Arsenic (as As)	3 ppm maximum	Nikitakis and McEwen 1990a
Lead (as Pb)	20 ppm maximum	Nikitakis and McEwen 1990a

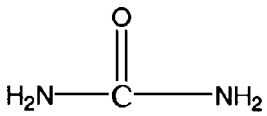

FIGURE 1
Formula for Urea (Wenninger et al. 2000).

into monomers when the number of water molecules became large enough.

Method of Manufacture

Commercially, Urea is synthesized from dry carbon dioxide and ammonia (Fleischman et al. 1980). Liquid ammonia and

liquid carbon dioxide at 1750 to 3000 psi and 160°C to 200°C react to form ammonium carbamate, which decomposes at lower pressure (~ 80 psi) to Urea and water. Several variations of the process include once-through, partial recycle and total recycle (Lewis 1993a). A large-scale process for preparing Urea involves heating calcium cyanamide with water under pressure (Gennaro 1990).

Analytical Methods

Main et al. (1987) used ^{1}H-nuclear magnetic resonance (NMR) to determine the amount of Urea in samples of known quantity. Tserng and Kalhan (1982) used gas chromatography/mass spectrometry to study the percentage of plasma [^{15}N]Urea in dogs formed from the infusion of L-[^{15}N]alanine. Shaya et al. (1978) used thin-layer chromatography and densitometry to determine the amounts of Urea present in human sweat. Urea was also analyzed by infrared (IR) spectroscopy (Nikitakis and McEwen 1990a).

Impurities

Urea contains not less than 99.0% and not more than 100.5% of CH_4N_2O (United States Pharmacopeial Convention, Inc. 2000).

USE

Cosmetic

The *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger et al. 2000) describes Urea as an organic compound used as a buffering agent, humectant, and skin-conditioning agent—humectant in cosmetic product formulations.

Raab (1990) stated that Urea may be incorporated into cosmetic preparations for the following reasons: moisturizing action, desquamating actions (Urea dissolves the intercellular cementing substance in the stratum corneum), antimicrobial action (the increased uptake of water interferes with the growth requirements of microorganisms) and buffering action.

Data submitted to Cosmetic Ingredient Review (CIR) by the Food and Drug Administration (FDA) based on industry reports in 2001 indicated that Urea was used in 239 formulations, which are described in Table 2 (FDA 2001). Current concentration of use data for Urea supplied by the cosmetics industry (CTFA 2000) are also presented in Table 2. A dash in any column means that no data were provided for that product category. Urea is reportedly used in some product categories for which the concentrations of use are not available. In other cases, information regarding use concentration for specific product categories is provided, but the number of such products is not known.

There are no restrictions for the use of Urea in cosmetics in Japan according to the Ministry of Health, Labor, and Welfare (2000), nor in Europe (European Economic Community 1999).

Noncosmetic

Urea is generally recognized as safe (GRAS) under 21 Code of Federal Regulations (CFR) 184.1923. Urea is approved under the following CFR cites for the following purposes: 21 CFR175.300 for use in side-seam cements for food contact; 21 CFR 182.99 when used as an inhibitor or stabilizer in pesticide formulations and formulations applied to animals; 21 CFR 178.3520 as internal sizing for paper and paperboard and as surface sizing and coating of paper and paperboard 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 free of fat or oil, and 21 CFR 240.1051 to facilitate fermentation of wine.

In a status report on over-the-counter (OTC) preparations, FDA indicated that Urea is present in hair grower, in-grown toenail and diuretic preparations, but that Urea is not generally recognized as safe and effective or is misbranded for these uses (FDA 1994).

According to Budavari (1989), Urea is a nutritional factor and antiseptic that promotes healing of infected wounds. Gennaro (1990) stated that urea is used intravenously (IV) as an osmotic diuretic for reduction of intracranial and intraocular pressure. Topical uses include treatment of psoriasis, ichthyosis, and atopic dermatitis, and to remove excess keratin from dry skin. It is used by transabdominal intra-amniotic injection as an abortifacient.

Urea is also used as a fertilizer, a chemical intermediate, a stabilizer in explosives, a diuretic, in animal feed, plastics, adhesives, sulfamic acid production, production of biuret and to separate hydrocarbons, flameproof agents, and modify viscosity in starch or casein-based paper coatings (Lewis 1993a).

GENERAL BIOLOGY

Grant (1972) described Urea as the end product of mammalian protein metabolism and the chief nitrogenous compound of urine.

Absorption

Battaglia et al. (1964) studied Urea absorption in monkeys. The tissue corresponding to the chorion laeve in the human placenta was obtained within 5 min of delivery from the placentas of healthy, pregnant rhesus monkeys. The amnion and chorion were also mounted for study separately. The gestational range for the animals in this study was 67 to 157 days. No additional details were available.

The mean Urea permeability constant for the chorioamnion, chorion and amnion was 16.0, 19.6 and 102.2 μl/min, respectively. No consistent changes in the permeability to Urea in each of these tissues was observed over the gestational range. The tissue resistivity of the chorioamnion, chorion and the amnion was 5905 ± 1450, 5500 ± 1731 and $66\,6 \pm 150$ s/cm^3. The chorion and chorioamnion were less permeable per unit weight to Urea than the amnion (Battaglia et al. 1964).

Hakim and Lifson (1964) used the mucosa membranes from the small intestine (jejunum and ileum) of the dog to study the

TABLE 2
Product formulation data

Product category (no. of formulations in category) (FDA 2001)	No. of formulations containing urea (FDA 2001)	Current concentration of use (CTFA 2000) (%)
Baby Products		
Baby shampoos (24)	—[a]	—
Bath Preparations		
Bath oils, tablets, and salts (140)	—	—
Eye Makeup		
Eye lotion (23)	2	0.1
Eye makeup remover (99)	2	—
Mascara (187)	3	8
Other eye makeup preparations (151)	5	—
Fragrance Preparations		
Colognes and toilet waters (683)	—	—
Hair Preparations (Noncoloring)		
Hair conditioners (630)	2	—
Permanent waves (211)	13	5–6
Rinses (noncoloring) (41)	1	—
Shampoos (noncoloring) (851)	6	3–10
Tonics, dressings, and other hair-grooming aids (577)	1	—
Wave sets (53)	2	5
Other hair preparations (276)	1	—
Hair Coloring Preparations		
Hair dyes and colors (1588)	18	4
Hair tints (49)	30	—
Hair bleaches (115)	—	1
Makeup		
Foundations (319)	4	—
Lipstick (942)	—	—
Makeup bases (136)	—	—
Rouges (16)	—	—
Other makeup preparations (186)	—	—
Nail Care Preparations		
Cuticle softeners (19)	1	—
Nail creams and lotions (15)	2	—
Oral Hygiene Products		
Dentifrices (38)	—	—
Mouthwashes and breath fresheners (46)	—	—
Personal Cleanliness Products		
Bath soaps and detergents (405)	—	2
Deodorants (underarm) (247)	2	2–8
Douches (5)	—	—
Other personal cleanliness products (307)	6	—
Shaving Preparations		
Other shaving preparation products (61)	1	2
Skin Care Preparations		
Cleansing (733)	11	1
Depilatories (28)	1	4
Face and neck (excluding shaving) (304)	16	3–5
Body and hand (excluding shaving) (827)	15	0.3–8
Moisturizing (881)	41	0.03–0.8
Night (200)	19	0.01–0.8
Paste masks (mud packs) (269)	8	—
Skin fresheners (181)	3	1
Other skin care preparations (715)	18	7
Suntan Preparations		
Suntan gels, creams, and liquids (131)	3	—
Indoor tanning preparations (68)	—	—
Other suntan preparations (37)	1	—
Total uses/ranges of urea	239	0.01–10

[a] A dash means that no data were provided for that product category.

in vitro transport of Urea across these membranes. The membranes were tied to one end of a glass cylinder 6.4 cm^2 in cross-sectional area and placed in a bath of 200 ml of "outer fluid." The inner compartment had the membrane-covered cylinder and a known volume of about 5 ml fluid added. A short time later about 3 ml of fluid was removed from the inner compartment, weighed, and saved for analyses. The final inner fluid volume was measured 1 h later. The serosal and mucosal surfaces were both exposed to the inner fluid during the experiment. The fluid was a Krebs-Ringer bicarbonate that contained one-half the prescribed calcium concentration and 500 mg/100 ml glucose. When the inner fluid was serosa, a correction for changes in Urea content of the nonepithelial portion of the membrane compartment was applied to the changes in volume and concentration of the inner fluid. This correction was not necessary when the inner fluid was mucosa. The rate of water transport per membrane in ml/hr was denoted by J_w and the rate of Urea transport in μM/h by J_u.

The downhill movement of Urea (mucosal fluid to serosal fluid) at initial Urea concentrations of 10, 20 and 50 mM in the mucosal fluid and 0 mM Urea in the serosal fluid resulted in the corrected values of 101%, 106% ± 3% and 128% ± 5% of Urea transported across these membranes relative to the mucosal fluid concentration. At the lower values for J_w more Urea is absorbed than can be accounted for by simple bulk flow. However, with increasing J_w, the concentration of Urea in the water progressively approaches simple bulk flow. When the initial serosal fluid concentrations were equal to the initial mucosal fluid concentrations of 10 and 20 mM, the corrected values were 96% ± 2% and 106% ± 2% of Urea transported across these membranes, respectively. During the uphill movement of Urea, the outer mucosal fluid contained 10 mM Urea and the serosal fluid contained 20 mM Urea. Urea moved in the direction of water transport, uphill from low to high concentration. The serosal Urea concentrations remained higher than the mucosal ones throughout the absorption period. At lower values for J_w, less Urea moved than provided for by simple bulk flow which is consistent with a back-diffusion component. As J_w increased, the Urea concentration rose toward the simple bulk flow line until, at J_w greater than 3 ml/h, it reached more than 90% of the *cis* concentration (the bathing fluid from which the water is leaving).

Labeled water (D_2O) was added to the outer mucosal fluid to a concentration of about 1%. The inner serosal volume was 1 ml. The glucose concentration in the bathing fluids varied from 0 to 800 mg/100 ml. A correction for D_2O accumulation in the membrane was applied as it was for Urea. The normal absorptive direction of water transport was reversed by excess hydrostatic pressure on the serosal side. The downhill movement of Urea from a serosal fluid concentration of 20 mM to a mucosal one of 10 mM approached simple bulk flow. Uphill movement of Urea from 10 mM Urea in the serosal fluid to 20 mM Urea in the mucosal fluid caused Urea to move into the mucosal fluid (Hakim and Lifson 1964).

Faber and Hart (1967) used 17 New Zealand pregnant white rabbits (gestation 27 to 29 days) to study the transfer of Urea across the placenta. The maternal animal was placed in a bath filled with saline (36.5°C ± 0.2°C). The abdomen and uterus were opened and the fetus was removed. The fetal side of the in situ placenta was perfused with deoxygenated blood obtained recently from another animal. The perfusion circuit had a disc tonometer, a calibrated constant-flow pump, a cotton-wool filter, and an arterial electrode curvette in which the hydrostatic pressure and oxygen pressure of the inflowing blood were continuously monitored. The venous blood flowing from the placenta flowed through a similar venous electrode curvette, then back to the tonometer via an outflow meter. At least 30 min were allowed for equilibration after each change in rate of perfusion of the placenta, except for the transfer of water when 20 min was allowed for equilibration. Isotoptes of Urea were added to the blood in the disc tonometer before the start of perfusion. In some experiments tritiated water and sodium (^{22}Na) and [^{14}C]Urea and chlorine (^{36}Cl) were combined in the perfusate.

The relative increase in concentration in the maternal blood leaving the placenta was characterized by a "transport fraction," T. These values for water, Na^+, Cl^-, and Urea were 84 ± 4.6, 7.6 ± 1.1, 8.8 ± 1.4 and 13.7 ± 1.6, respectively. The dimensionless variable d is defined as $d = P/(Q_M f_M)$ where P is the permeability of the placenta and f is a factor that expressed the fraction of the blood volume in which the isotope can dissolve. Therefore, f equals one if the isotope distributes quickly and evenly over plasma and red cells (chloride, 9; Urea and water, 13). Sodium had a value of 2 for f and does not invade red cells. The value of d for water, Na^+, Cl^-, and Urea was 2.6, 0.083, 0.097 and 0.160, respectively. The value for the permeability of the placenta (P) was 5.5, 0.11, 0.21 and 0.34 ml/min for water, Na^+, Cl^-, and Urea, respectively (Faber and Hart 1967).

Ochsenfahrt and Winne (1973) studied the effect of water net flux (so-called solvent drag) on the intestinal absorption of Urea or tritiated water. Jejunal loops from male Wistar rats were perfused in vivo with buffered hypotonic, isotonic or hypertonic solutions that contained [^{14}C]Urea (12.64 ± 0.04 μM, specific activity 39 mCi/mmole, pH = 6.2) or tritiated water (10.96 ± 0.05 μCi/ml). The experiment involved three test periods of 10-min duration during which blood and perfusate were collected. Additional tests were performed with Urea to determine the effect of pH = 2.2 on absorption.

The intestinal epithelial permeability of tritiated water is 10 times larger than the permeability for Urea (pH = 6.2). The results of Urea absorption demonstrated the strong role of the osmolality of the solution. Taking the isotonic solution as the norm, the appearance rate of Urea in blood was 41% higher from the hypotonic solution and 32% lower from the hypertonic solution. The influence of reducing the pH of the solution was to considerably increase the appearance rate of all three solutions, with little change in the relationship among the three (Ochsenfahrt and Winne 1973).

Abramovich et al. (1974) performed three studies to determine the transfer of labeled Urea to the fetoplacental unit in

midpregnancy. The gestation sacs from two patients admitted for hysterotomy and sterilization were removed intact. The sac was then opened and the fetus immersed in a bowl containing 25 μCi of [^{14}C]Urea and 3 μCi chlorine-36. After 2 min, blood was collected from the cut ends of the umbilical cord. In the second experiment, the gestation sacs from two patients admitted for hysterotomy and sterilization were also removed intact. As the placenta was held up to form a container, 15 ml of liquor amnii with radioactive Urea in the doses described previously were added to the fetal surface of the placenta.

The experiment was terminated after 2 min and the radioactivity present in fetal blood and the liquor amnii was determined. Each of the previous experiments were repeated twice. In a third experiment, experiments were performed as described previously, but the radioactive substances were dissolved in 0.25 ml normal saline and injected directly into the umbilical artery. The liquor amnii (15 to 20 ml) was placed on the fetal surface of the placenta with the membranes held as described previously. The dose of [^{14}C]Urea injected was 25 μCi. Both liquor amnii and fetal blood were collected after the experiment.

The fetuses used in the first experiment (the transfer of radioactivity from liquor amnii to fetal circulation across fetal skin) were 13 and 17 gestational weeks. The specific activity of the liquor amnii was 31×10^5 and 22.6×10^5 dpm/ml for the 13- and 17-week fetuses, respectively. For the same two fetuses, the radioactivity of fetal plasma was 1665 and 1170 dpm/ml, respectively. The fetuses used in the second experiment (the transfer of radioactivity from liquor amnii to fetal circulation across the fetal surface of placenta) were 15 and 16 gestational weeks. The specific activity of the liquor amnii was 11.9×10^5 and 32×10^5 dpm/ml for the 15 and 16 week fetuses, respectively. For the same two fetuses, the radioactivity of fetal plasma was 3812 and 1656 dpm/ml, respectively.

The fetuses used in the third experiment (transfer of radioactivity from fetal circulation to liquor amnii across the fetal surface of the placenta) were 16 and 17 gestational weeks. The specific activity of fetal plasma was 37.2×10^5 and 39.9×10^5 dpm/ml for the 16- and 17-week fetuses, respectively. The radioactivity of liquor on fetal plate was 5886 and 4300 dpm/ml, respectively (Abramovich et al. 1974).

Okumura et al. (1975) ligated the efferent ducts of male rats 18 to 20 h prior to cannulation of the rete testis. A stable flow rate, which ranged from 30 to 90 μl/h/testis, of rete testis fluid was achieved 60 to 90 min after cannulation. Rete testis fluid was collected at 15-min intervals. Plasma concentrations of [^{14}C]Urea (61 mCi/mmol) were established and maintained constant by a combination of priming injections and continuous iv infusion. [^{14}C]Urea was slowly transported into the rete testis fluid and approached the unbound plasma concentration by 120 min. The concentration ratio of rete testis fluid to plasma (unbound) 20 min after the start of infusion was 0.5. The transfer rate of Urea was 0.030 ± 0.005 per minute.

Theodore et al. (1975) used mongrel dogs of both sexes to study the transport of Urea in the saline filled lung. This study was performed on intact dogs that had been anesthetized with sodium pentobarbital. Simultaneous blood and alveolar liquid samples were obtained at 0, 60, 120, 180, and 240 min. The rate of accumulation of Urea from plasma to alveolar liquid was determined by measuring the rate of accumulation in the alveolar liquid. Urea presumably entered the alveolar water by penetration of the capillary surface and the alveolar surface of the alveolar epithelial cells. Urea had a mean $T_{1/2}$ of 1.2 ± 0.17 h and an apparent capillary and alveolar permeability constant (P′) of $2.4 \pm 0.28 \times 10^{-6}$ cm/s. The investigators used values from coefficients of heart muscle capillary and the values they obtained for the total barrier permeability coefficient and determined the calculated values ($\times$ cm^{-1}) for pulmonary capillary resistance, alveolar epithelium resistance and total barrier resistance as 3.2×10^4, 3.8×10^5 and 4.2×10^5.

Bissonnette et al. (1979) studied placental transfer of [^{14}C]Urea in pregnant guinea pigs between 55 and 65 days of gestation. A bolus of 10 to 20 μCi of [^{14}C]-Urea was mixed with 15 to 25 μCi of tritiated water in 0.8 ml normal saline and rapidly injected into a catheter placed in the left ventricle or the descending thoracic aorta above the diaphragm. (No difference in placental transfer was measured with regard to the position of the catheter.) Blood was drawn from the fetal umbilical vein 0 to 25 s after the bolus injection (this time was determined based on previous studies). The erythrocyte permeability for Urea was 23.87×10^{-5} cm/s and the placental transfer index (PTI) was 0.18 ± 0.01. The slope of the relationship between placental transfer and lipid solubility was 0.279 to 0.336. These values are based on four experiments.

Dancis et al. (1981) perfused four, fresh human placentas from uncomplicated term gestations with [^{14}C]Urea. The transplacental gradient was 6 and 20 mg/dl. The perfusates were not recirculated. The respective mean clearance indexes of Urea from fetus to mother and from mother to fetus was 0.38 and 0.32 (the doses for these results were not specified). These values were not considered statistically significant. In another experiment, the maternal perfusate contained 20 mg/dl Urea with a trace of [^{14}C]Urea infused during the first 30 min and the fetal perfusate contained no Urea. During the second 30 min of perfusion, the maternal perfusate was retained unchanged and 20 mg/dl Urea was added to the fetal perfusate. The clearance index under conditions of zero gradient was the same as the clearance index with the gradient of 20 mg/dl. According to these results, Urea was transferred across the placenta by simple diffusion.

Yuasa et al. (1997) determined the effects of aging on the intestinal transport of Urea perfused as a single pass through the small intestine of male Wistar rats at 8, 54 and 101 weeks of age. Anesthetized rats were perfused with unlabeled compound and a trace amount of [^{14}C]Urea in combination with the nonabsorbable marker [^{14}C]inulin. The outflow solution was collected for four 5-min intervals 25 min after initiation of perfusion. Rat small intestine (jejunum to midgut) everted sacs were used in uptake experiments. The everted sacs were incubated with

unlabeled Urea plus trace amounts of labeled Urea in combination with the nonabsorbable marker, PEG 4000.

Urea did not demonstrate an age dependency in membrane permeability clearance at 54 and 101 weeks. The membrane permeability clearance for the 8-week-old rats was a little greater than the values for the other two ages. Although a statistical analysis was performed, the investigators did not assign a statistical significance to this value. Likewise, experiments with the everted sacs did not demonstrate changes in the intestinal uptake of Urea based on the age of the rat (Yuasa et al. 1997).

Distribution

Luck and Engle (1929) injected Urea subcutaneously into 24 rats on day 18 or 19 of gestation. Urea was dissolved in 5 cc of 0.9% sodium chloride and the equivalent of 0.4 g nitrogen/kg was given to the rat. Animals were killed and the uterus and several fetuses rapidly excised from 15 min to 7 h after injection. The Urea concentration in muscle, liver, and fetus had become greater than or equal to the normal state within 2 h of dosing. The investigators stated that Urea diffuses readily through the placenta and into other maternal and fetal organs.

Hutchinson et al. (1962) placed catheters in term pregnant rhesus monkeys in the maternal femoral artery, an interplacental vessel and the amniotic artery. In all cases, live baby monkeys were obtained 3 to 12 days after the operative procedures. [^{14}C]Urea was injected into the maternal vascular tree, the fetal circulation and the amniotic fluid in three separate experiments. The amount of [^{14}C]Urea injected in these experiments was 5.9 mg.

When [^{14}C]Urea was injected into the maternal vascular tree, the concentration of [^{14}C]Urea present in fetal and maternal blood and amniotic fluid after 200 min was 16.2 and 22.0 mg/100 ml, respectively (control values were 16.6, 15.9 and 20.5 mg/100 ml for fetal blood, maternal blood and amniotic fluid). Injection of [^{14}C]Urea into fetal circulation caused [^{14}C]Urea concentrations of 41.0 and 45.0 mg/100 ml in fetal and maternal blood, respectively, after 190 min (values were not available for amniotic fluid). Control values were 44.1, 45.1 and 52.0 mg/ 100 ml, respectively, for fetal and maternal blood and amniotic fluid. Injection of [^{14}C]Urea into amniotic fluid produced [^{14}C]Urea concentrations of 17.3, 20.6, and 36.0 mg/100 ml in fetal and maternal blood and amniotic fluid, respectively, after 215 min (Hutchinson et al. 1962).

In another experiment reported by Hutchinson et al. (1962), two Urea tracers ([^{13}C]Urea and [^{14}C]Urea) were injected simultaneously; 125 and 147 mg [^{13}C]Urea were injected into the maternal blood stream (experiment 1) and 5.9 mg [^{14}C]Urea was introduced into fetal circulation (experiment 2). Samples from the amniotic fluid and fetal and maternal blood were collected for 1 h. The number of monkeys per group was not available. The weights of the mothers in experiment 1 and 2 were 4.56 and 6.55 kg, respectively, while the weights of the fetuses were 0.435 and 0.380 kg, respectively. The volume of amniotic fluid in experiments 1 and 2 was 100 and 80 ml, respectively. The concentration of Urea in maternal blood was 27.0 and 21.4 mg/100 ml in experiment 1 and 2, respectively. The concentration of Urea in fetal blood was 24.1 and 26.3 mg/100 ml in experiment 1 and 2, respectively. The concentration of Urea in amniotic fluid was 33.0 and 30.9 mg/100 ml in experiment 1 and 2, respectively.

Gregoire and Rakoff (1963) used sexually mature New Zealand rabbits (5/group) to study the Urea content in female genital tract fluids. Intact animals were ligated for 72 h at the cervical and tubal end of the uterus and uterine and ovarian portion of the Fallopian tubes with surgical thread. The remaining does were spayed and rested for 7 days before ligation. Estradiol benzoate (5 μg), progesterone (1 mg) or a combination of both was administered daily intramuscularly (IM) for 3 days. At the end of this period the animals were killed and the complete genital tract was removed. The fluid-filled tract was held over a small funnel and the fluid was allowed to flow through a small puncture.

The average amount of Urea in uterine and tubal fluid of intact animals was 43.5 and 53.1 mg/100 ml, respectively. Spaying resulted in a decrease in Urea concentration to 21.6 and 28.2 mg/100 ml in uterine and tubal fluid, respectively. A statistically significant increase in Urea content of tubal fluid was observed in animals that received estrogen. No significant change in tubal fluid Urea content was observed in animals that received progesterone or progesterone and estrogen. However, administration of estrogen or progesterone caused a significant increase in Urea concentration of uterine fluid. The combination of estrogen and progesterone did not cause a significant interaction, positively or negatively, with respect to Urea concentration in uterine fluid. Therefore, the effect of estrogen and progesterone is to increase the concentration of Urea independently (Gregoire and Rakoff 1963).

Regoeczi et al. (1965) conducted isotopic studies of Urea metabolism in rabbits. Seven male Sandylop rabbits received intravenous injections of a mixture of [^{14}C]Urea (2 to 6 μCi) and [^{15}N]Urea (12 to 20 mg) together. Four rabbits received [^{14}C]Urea through a rubber catheter into the emptied bladder. Blood samples were taken at intervals for estimating Urea specific activities. Two rabbits received IV injections of [^{14}C]Urea to observe plasma specific activities with and without catheterization.

The diffusion of Urea from the body pool into the bladder were investigated in one rabbit by ligating the ureter just below the level of the kidneys before injecting [^{14}C]Urea. The [^{14}C]Urea was allowed to diffuse into the bladder for 1 h. The bladder also contained a solution of unlabeled Urea, which corresponded in volume, Urea mass, and concentration to a 4-h urine sample from a normal rabbit. Two rabbits were injected with 4.31 μCi and 5.09 μCi [^{14}C]Urea and ^{51}Cr-labeled erythrocytes and killed 4 to 5 h later to measure Urea and water in both kidneys. Body water mass and distribution spaces of Urea

were compared in five rabbits using [^{14}C]Urea and HTO water at different times.

Ten normal rabbits were injected with [^{14}C]Urea and blood samples were taken at regular intervals. Quantitative urine collection by catheter was taken at intervals over 8 h. Blood samples in these experiments were taken over a period of 6 to 10 h and quantitative catheterizations were performed 2 to 3 min before each blood sample was collected.

The ^{14}C specific activities and ^{15}N enrichments were both higher after 30 min in the urine than in the plasma, urine/plasma ratios were slightly higher for ^{14}C than ^{15}N in the seven rabbits injected with a mixture of [^{14}C]Urea and [^{15}N]Urea together. The half-life of [^{15}N]Urea was 3.05 h and the half-life of [^{14}C]Urea was 2.50 h. No linear correlation was found between Urea catabolic rates and amount of recycling of nitrogen. The values from the slopes of terminal plasma specific activity exponential (k^{15}N/k^{14}C) were from 0.69 to 0.84 for all seven rabbits. The differences in synthesis rate (Urea pool $\times$ k) for the seven rabbits were 5.5 to 12.0 mg/h. The reutilization rate of Urea nitrogen (differences in synthesis rate/Urea catabolism rate measured with ^{14}C) were 32.2% to 88.8%. Rabbit number 7 also had data from 6 and 31 days after the [^{14}C]Urea/ [^{15}N]Urea combined injection and received a treatment of neomycin sulfate (4×0.5 g orally) over 40 h. The specific activity exponential, difference in synthesis rate, and reutilization rate of Urea nitrogen at 6 days was 0.91, 2.9 mg/h, and 22.8%, respectively. The values at 31 days were 0.89, 3.5 mg/h, and 17.7%, respectively.

The first blood sample taken 10 min after Urea was introduced into the urinary bladder of four rabbits indicated the presence of significant radioactivity. Extrapolation of values over the first hour indicated that about 5% of the dose had passed from the urinary bladder into the body Urea pool. However, the animal with ligated ureter that was dosed with [^{14}C]Urea IV had 0.01% of the Urea transferred in 1 h. The investigators stated that the reabsorption of labeled Urea from the bladder might be decreased by frequent catheterization. An abrupt change in slope, coinciding with the onset of catheterization, suggested that the synthesis rate for Urea was increased by 14.5% and no change occurred in the plasma Urea concentration or in Urea output.

The distribution of Urea in the kidneys of two rabbits was also determined. The total renal Urea was 13.71 mg and 8.52 mg, respectively, of which >1% was attributed to trapped blood. The Urea excretion rate was 30.4 and 26.6 mg Ci/h, respectively. The specific activities were slightly lower in the renal cortex than in the plasma at the same time. The Urea content, concentration and ^{14}C specific activity of Urea were all highest in the renal medulla.

Other parameters determined were: the total body water volume (65% to 69%); excretion rates (varied from 8.2 to 38.4 mg/h); and delay time variations (0.33 to 1.05 h). The maximum rate of expiration after injecting [^{14}C]Urea occurred at 2 h. The Urea carbon space, as a percent of body weight, varied from 43% to 64%. One rabbit treated with neomycin increased the excretion of Urea from 0.4 to 0.57 (units not defined). Additionally, the renal pool was increased from 4.1 to 14.7 mg of Urea carbon and the values for excess excretion increased proportionately (Regoeczi et al. 1965).

Bourne and Barber (1972) administered 1 to 4 μCi/animal [^{14}C]Urea alone or with unlabeled material (10 or 20 mg/100g) by rapid IV injection to three male Wistar rats. The rats were anesthetized and the trachea, jugular vein, carotid artery, and both ureters were cannulated. Heparin was injected IV (125 IU/100 g) and a 6% solution of mannitol in 0.9% saline was infused into the jugular vein at 0.075 ml/min. Blood samples were taken at 1, 2, 3, 5, 8, 12, 15, 20, 25, 35, 45, and 55 min after injection and urine was collected from the ureter at 2-min intervals from 0 to 10-min, then for five 10-min intervals.

Although a good fit of the plasma data by a biexponential function was obtained, the authors stated that a two-compartment model could not predict both urine and plasma results because the experimental fraction eliminated was above or below the levels predicted from the plasma data. The fractions eliminated as predicted by a two-compartment model from the analysis of the biexponential function was a poor fit to the experimental fractions eliminated from both individual and mean results. A triexponential function fitted to the plasma data and analyzed by the three compartment model better described the experimental results. Elimination of Urea in bile accounted for only 0.5% of the dose and was determined to be a minor route of elimination. Plasma concentration and urine elimination data are given in Tables 3a and 3b, respectively (Bourne and Barber 1972).

Blake et al. (1976b) injected three mid-gestation rhesus monkeys directly into the amniotic fluid with [^{14}C]Urea (58% *w/v*, 2.3 g/kg, 25 μCi). Amniotic fluid and blood were obtained before and at 7.5, 12.5, 15, 22.5, 45, 90, 150, 210, 480, 720, 960, and 1440 min after injection of labeled Urea. Urine was collected by catheter at 0, 15, 30, 60, 120, 180, and 240 min.

TABLE 3a
Plasma concentrations of Urea (Bourne and Barber 1972)

	Plasma concentration (nCi radiolabel/ml)			
Time (min)	Rat 1	Rat 2	Rat 3	Average
1	15.7	13.6	16.3	15.2
2	13.4	12.3	10.9	12.2
3	11.2	10.5	9	10.2
5	7.8	8.3	7	7.7
8	8.9	6.8	6	7.2
12	5.8	5.8	5.3	5.6
15	6	5.3	4.9	5.4
20	5.6	5.1	4.7	5.1
25	4.7	4.8	4.5	4.7
35	4.2	4.5	4.1	4.3
45	4.1	4.5	4	4.2
55	4.2	4.2	3.8	4.1

TABLE 3b
Urea elimination in urine (Bourne and Barber 1972)

Cumulative fraction eliminated in urine				
Time (min)	Rat 1	Rat 2	Rat 3	Average
10	0.05	0.04	0.04	0.04
20	0.1	0.08	0.08	0.09
30	0.13	0.11	0.12	0.12
40	0.16	0.13	0.15	0.15
50	0.18	0.16	0.18	0.17
60	0.19	0.17	0.21	0.19

TABLE 4
Pharmacokinetics of three-compartment fit of urea concentration and excretion data (Blake et al. 1976b)

Compartment variables	Average[a] (h^{-1})
K_{am} (amniotic fluid ⇀ maternal water)	0.018
K_{ma} (maternal water ⇀ amniotic fluid)	0.027
K_{af} (amniotic fluid ⇀ fetal water)	1.15
K_{fa} (fetal water ⇀ amniotic fluid)	1.092
K_{fm} (fetal water ⇀ maternal water)	0.318
K_{mf} (maternal water ⇀ fetal water)	0.042
K_u (maternal water urine)	0.2
Pregnancy duration	76.3 days

[a] Average of three monkeys; parameters of monkey no. 1 did not represent a least-squares fit because of incomplete urine collection.

The rate of apparent volume expansion was 5 ml/h for all three animals. Ultrafiltration of amniotic fluid containing ^{99m}Tc-labeled albumin ([^{99m}Tc]albumin) revealed that 6% to 10% of the radioactivity was unbound and could have moved out of the amniotic space. The biphasic exponential decay in amniotic fluid Urea concentration had a $t_{1/2}$ of 24 min in the initial phase and the later phase had a $t_{1/2}$ of 7 h. The investigators assumed that the initial rapid phase represented passage into fetal and placental tissue and the slower phase represented transfer to maternal water. The time of peak serum [^{14}C]Urea was 4 to 6 h after injection of labeled Urea.

From the curve that fit the peak serum data the investigators suggested that (1) the Urea concentrations in the fetal compartment rise rapidly and peak at about 1 to 1.5 h, and that (2) although an equilibrium is achieved between amniotic fluid and fetal Urea concentration after 2 h, the concentration in the fetal compartment remained below that in amniotic fluid even though the apparent rate constant into the fetus is larger than from the fetal to maternal compartment. The investigators suggested that this observation was due to blood flow limitations between the fetus and placenta. This explanation would also account for the greater differential between Urea concentrations in the fetal and maternal compartments. About 3% of the Urea that entered the maternal compartment came directly from the amniotic fluid; most of the [^{14}C]Urea dose arrived via the fetal compartment. The rate constant for transfer of Urea from the amniotic fluid to the fetal compartment was independent of the concentration in amniotic fluid. Excretion data are presented in Table 4 (Blake et al. 1976b).

Thornburg and Faber (1977) ligated the branch point of the vitelline vessel of pregnant guinea pigs 1 to 20 days before the experiment. A sham-operated littermate had the same surgery, but a suture was placed on the surface of the yolk sac adjacent to the blood vessel. Unoperated littermates and fetuses from unopened sows served as controls. Catheters were placed in a jugular vein and carotid artery the 1st or 2nd day after ligation of the yolk sac vessels. [^{14}C]Urea and/or another tracer was injected into the maternal jugular vein at time 0. Maternal arterial blood was sampled from the carotid artery at regular intervals for about 10 min. The placental permeability of ligated vitelline vessels vs. intact vitelline vessels ($1.2 \times 10^{-3} \pm 3.8 \times 10^{-4}$ versus $1.0 \times 10^{-3} \pm 2.7 \times 10^{-4}$ ml/g/s, $N = 5$) were not significant. The permeability of chorioallantoic placenta of the guinea pig as a function of molecular dimension was $1.1 \times 10^{-3} \pm 2.1 \times 10^{-4}$ ml/g/s ($N = 8$). The coefficient of free diffusion, $D_w^{38.5}$ was 1.92×10^{-5} cm^2/s.

Davis et al. (1978) infused pregnant rats from the Wistar strain (2 litters) with Urea-1-^{14}C (3.7 μCi/μM, total infused = 10 μCi). The right carotid artery was cannulated to obtain maternal blood samples and the femoral vein was cannulated to infuse Urea at an initial rate of 0.1 ml/min for 10 min and then 0.01 ml/min for the remainder of the experiment (3 h). The average accumulation of Urea-1-^{14}C in the near term pregnant rat was: fetal to maternal plasma ratio of 0.79, the fetal labyrinth to maternal plasma of 0.80, the visceral yolk sac to maternal plasma of 0.70, and the maternal-fetal junctional zone to maternal plasma of 0.84.

Johanson and Woodbury (1978) injected (intraperitoneal) nephrectomized Male Sprague-Dawley rats with [^{14}C]Urea (45 μCi, 44 rats) or [^{3}H]water (5 μCi, 12 rats). Eight hours after injection, [^{14}C]Urea attained a steady-state distribution in 78% of the tissue water of the lateral ventricular choroid plexus and about 8 h was required for labeled Urea to reach a steady-state concentration in the cerebral cortex and cerebrospinal fluid (CSF). Although the concentration of labeled Urea in choroid cell water increased after 3 h, it remained in equilibrium with the concentration of [^{14}C]Urea in the CSF water. At the steady state, labeled Urea distributes into 88% of the water of the cerebral cortex. The investigators concluded that Urea movement is hindered at the blood-brain barrier as well as the blood-CSF barrier.

Rapoport et al. (1982) conducted a study in which male Osborne-Mendel rats were either infused IV for 10 (two rats), 20 (three rats), or 40 (three rats) min with 10 μCi of [^{14}C]Urea or given a bolus IV injection of [^{14}C]Urea and killed at 10, 20, and 30 min after injection. Brain radioactivity was determined at the end of infusion when the rats were killed. Chemical separation

procedures were not required to distinguish [^{14}C]Urea from a radioactive intermediate in any tissue, and tissue radioactivity was assumed to equal [^{14}C]Urea concentration because Urea is not metabolized by the rat. The following regions of the brain had approximately equal concentrations of radioactivity after a bolus injection or continuous infusion of [^{14}C]Urea for up to 40 min: frontal lobe, caudate nucleus, hippocampus, thalamus plus hypothalamus, pons, and white matter (corpus callosum).

Challier et al. (1985) collected human placentae after delivery from 3 to 14 subjects. Fetal perfusion started 30 min after delivery. Afterwards, the whole placenta was used and two glass cannulae were introduced into the intervillous space to initiate the perfusion on the maternal side. The medium was not recirculated. Maternal-fetal transfer was studied using [^{14}C]Urea (40 to 50 mCi/mM) and Urea plus tritiated water (0.47 mCi/mM). Additional details were not available. The maternal flow rate of Urea-$^{3}H_2O$ was 22 ± 3.80 ml/min, the ratio of fetal to maternal flow rates of Urea-$^{3}H_2O$ was 0.32 ± 0.092, the clearance of first molecule of Urea-$^{3}H_2O$ was 1.40 ± 0.38 ml/min, the clearance of the second molecule of Urea-$^{3}H_2O$ was 2.87 ± 0.87 ml/min and the clearance index of Urea-$^{3}H_2O$ was 0.49 ± 0.015.

Hisanaga et al. (1991) conducted a study in which the brush border membrane vesicles were separated from five human placentae obtained immediately following full-term deliveries. The amount of [^{14}C]Urea transported across the brush border membrane vesicles in vitro was measured by rapid membrane filtration. Urea transport was started by the addition of 20 μl of brush border membrane vesicles to 130 μl of 1 mM [^{14}C]Urea. The mechanism of [^{14}C]Urea transport by reciprocal transport of H^+ occurred in human placental brush border membrane. Additionally, the transport of [^{14}C]Urea into the brush border membrane vesicles was dependent on the H^+ concentration gradient. The concentration of [^{14}C]Urea was varied from 1 to 20 mM to examine changes in the initial velocity of transport (the amount transported after 20 sec). The values for K_M and V_{max} were obtained from this data and determined to be 10.8 mM and 410 μmol/mg protein/20 s, respectively.

Metabolism

Wolfe (1981) measured Urea kinetics in vivo in eight dogs using a [di-^{15}N]Urea tracer. The dogs were fasted 18 h prior to experimentation. Two dogs had their livers surgically removed to eliminate the production of endogenous Urea. Urea was infused at known rates as soon as the livers were removed. Six dogs, with urinary catheters, were evaluated in two groups of three. One group was anesthetized to insert the catheter and the other group was operated on to insert the catheter and was allowed to recover while being treated orally with 500 mg/day ampicillin for 7 days to exclude hydrolysis of Urea in the gut. The infusion rates for [di-^{15}N]Urea were between 0.2 and 0.4 μmol/kg/min and 25000 and 35000 dpm/kg/min for [^{14}C]Urea. Additional unlabeled Urea was also infused in all experiments. The two hepatectomized dogs were infused with unlabeled Urea at a rate of 10 μmol/kg/min for 120 min, then the infusion rate was doubled for 60 min and then returned to the 10 μmol/kg/min. All experiments with intact animals included a 3-h basal period, during which only isotopes were infused. During the next 1 to 2 h, unlabeled Urea was infused at rates two- to fourfold in excess of endogenous Urea followed by another hour of only isotope infusion. Arterial blood samples were collected at intervals of 5 to 15 min starting after 90 min of isotope infusion. The unlabeled Urea infusions were based on the estimated basal Urea production.

In each hepatectomized dog, the total Urea production (R_{aT}) was 10 μmol/kg/min. Because the liver was removed and recycling was eliminated, R_{aT} is equal to Urea produced from nonrecycled nitrogen (R_{aN}). In the three dogs that remained anesthetized, an average of $12.9\% \pm 2.3\%$ of Urea nitrogen was recycled into Urea and $5.1\% \pm 3.2\%$ of Urea nitrogen was recycled into protein. Urea oxidation accounted for $13.0\% \pm 3.0\%$ of R_{aT} (^{14}C). Dogs allowed to recover and treated with antibiotics did not recycle Urea nitrogen, as less than 1% of R_{aT} was oxidized. The investigators stated that assuming a volume of distribution of 100 ml/kg in all experimental circumstances enabled them to estimate the true rate of Urea production within $\pm 20\%$ (Wolfe 1981).

Kalhan et al. (1982) measured Urea and glucose kinetics in 10 normal subjects (34.5 ± 2.9 weeks' gestation), 3 gestationally diabetic subjects (35.6 ± 2.9 weeks' gestation), and 7 juvenile-onset insulin-dependent diabetic subjects (36.7 ± 4.9 weeks' gestation). A bolus prime of 2 mg/kg [$^{15}N_2$]Urea was infused followed by a constant infusion of 0.2 mg/kg/h for 5 h. An initial prime was not necessary for the [6,6-$^{3}H_2$] glucose tracer that was infused at the rate of 30 μg/kg/min. Both tracers were infused IV into the superficial vein of the forearm. Blood samples were drawn at 30-min intervals. Timed urine samples were collected from all subjects throughout the study period.

A steady state of ^{15}N and ^{2}H enrichments of plasma Urea and glucose was achieved between the second and third hour of tracer infusion in pregnant subjects. A steady-state [$^{15}N_2$]Urea enrichment was observed after the second hour, although a prime injection had been administered. Both substrate concentration and isotopic enrichments were unchanged during the 4th and 5th hour in pregnant subjects. No significant differences between normal and diabetic subjects in the plasma Urea concentration and Urea synthesis rates were observed antepartum. About 90% of the Urea synthesized was excreted in the urine in the three groups. The remaining 10% was either metabolized or excreted by extrarenal mechanisms. The rates of ammonia and total nitrogen excretion in the urine were also similar between the normal and diabetic subjects.

The effect of pregnancy on nitrogen and glucose metabolism was also evaluated in three normal, three insulin-dependent diabetic subjects, and two gestationally diabetic subjects. Plasma Urea nitrogen and glucose concentration were increased significantly in the postpartum period. A rise in plasma Urea nitrogen was associated with a similar significant increase in the rate of

Urea synthesis. Pregnancy did not have an effect on the metabolized component of Urea turnover. Urea constituted about 80% of the total nitrogen excreted in the urine during pregnancy and postpartum (Kalhan et al. 1982).

Hewitt and Nicholas (1983) conducted a study in which the lungs of adult female hooded Wistar rats were excised and perfused (first group of rats). The perfusate was changed to 0.2, 0.5, or 1.0 mM Urea and 2 μCi [^{14}C]Urea (59 mCi/mmol) after 10 min of equilibration. The Urea and labeled Urea perfusate was recirculated. In a separate group of experiments, the ureolytic activity of rat isolated lung preparations were investigated. The perfusate contained 0.5 mM Urea and micromolar concentrations of the urease inhibitor *dl*-methionine hydroxamic acid and 1 mM fluoride ion. A third group of rats had 50 μg/ml neomycin added to their drinking water for three days which was equivalent to an oral dose of 600 mg/kg/day. A control group was established in which the absence of pulmonary bacterial urease was assured. This group was created by adding 50 μg/ml neomycin and 0.5 mM Urea and labeled Urea to the perfusate of isolated lung preparations from the rats in the third group.

Lungs perfused with Urea concentrations ranging from 0.2 to 1.0 mM had a $^{14}CO_2$ production that was linear with respect to time except for the last 20 min. Rat lung urease had a K_M = 5.6 mM, an apparent V_{max} = 17 nmol/min in the first group of rats and a correlation coefficient for the Lineweaver-Burk plot was 0.9997. The value of V_{max} suggested that the urease activity in the lung had no influence on the whole body clearance of Urea. The enzyme was inhibited by fluoride ions and *dl*-methionine hydroxamic acid. The I_{50} for the enzyme was estimated as 4.3 μM and the maximum inhibition achieved was 56% at 5 μM hydroxamic acid. One millimolar fluoride inhibited ureolysis 97%. Urea hydrolysis was unaffected when rats were retreated chronically with neomycin and when the antibiotic was present in the perfusate (Hewitt and Nicholas 1983).

Deutz et al. (1998) evaluated soy and casein as a source of protein in 12 female crossbred pigs. [^{15}N-^{15}N]Urea was used as a tracer to measure Urea kinetics in the gut, liver and muscle. The animals had multiple catheters implanted 2 to 3 weeks prior to study initiation. Food was removed the day before the experiment. On the morning of the experiment, an infusion of 25 mmol/L-*para*-aminohippuric acid (PAH) was made through the portal infusion catheter and continued throughout the experiment. Valine and phenylalanine were infused immediately after the PAH via the inferior caval vein catheter. A primed (135 μmol/kg body weight) constant, continuous infusion of 15 μmol/kg/h [^{15}N-^{15}N]Urea was given via the same catheter. Initial blood samples were taken one hour after the start of a constant and continuous infusion of the liquid test meal infusions via the gastric catheter. Blood samples were taken again 2 h after the start of enteral nutrition. The infusion of valine, phenylalanine and PAH were stopped. The infusion of [^{15}N-^{15}N]Urea and the enteral meal was continued for 6 h after the start of the meal infusion.

Whole body Urea production (R_{aT}) was not higher at 2 h. At 6 h, R_{aT} was higher during the infusion of soy protein meal infusion compared to casein protein meal infusion. Enteral nutrition increased the amount of Urea recycling relative to whole body Urea production. Recycling of Urea did not differ significantly between the two protein sources infused (Deutz et al. 1998).

Excretion

Houpt (1963) fed male New Zealand rabbits (seven animals total), at least 6 months old, a low-protein, carbohydrate-supplemented ration for 10 to 20 days before each Urea exposure. The ration composition was 20 to 25 g wheat straw, 100 to 150 g carrots, 12 g sucrose, 3 drops cod liver oil, and 100 to 250 ml water. This ration caused a negative nitrogen balance in the animals and they slowly lost weight.

Rabbit "D" was injected IV with 860 mg Urea-nitrogen and over the next 24 h period 842 mg Urea-nitrogen was excreted in the urine, whereas 31 mg remained in the body water. The basal amount of Urea-nitrogen, the mean of the two pre- and postexperimental control days, excreted was 226 mg. Therefore, the Urea in excess of the basal amount, 616 mg, was assumed to be injected Urea. About 25% of the injected Urea-nitrogen, 219 mg, was presumably used in amino acid synthesis and retained.

In a total of eight experiments performed on rabbit "D" the average Urea-nitrogen used was 212 mg. No evidence indicated that Urea was excreted in the form of another nitrogenous compound.

Rabbits "B" and "F" were injected with 770 and 852 mg Urea-nitrogen, respectively, and excreted 755 mg and 834 mg Urea-nitrogen at 24 h. The basal rate, amount stored and amount used for rabbit "B" was 172, 14 and 176 mg, respectively. The basal rate, amount stored and amount used for rabbit "F" was 163, 50, and 142 mg, respectively.

Rabbit "R" was dosed with 949 mg Urea orally with water available for 12 days and had a urine and fecal output of 1161 and 128 mg Urea. Rabbit "R" was dosed with water only for 13 days and had urine and fecal outputs of 287 and 113 mg Urea. When rabbit "R" was dosed with 913 mg Urea and had no access to water for eight days, urine and fecal output was 1095 and 119 mg Urea. When water was withheld for 3 days and rabbit "R" was not dosed with Urea, the urine and fecal output was 278 and 98 mg Urea.

Rabbit "S" did not receive Urea, but did receive an antibacterial mixture of 38 mg sulfaquinoxaline plus 20 mg neomycin sulfate per day in two equal doses. Rabbit "S" formed a total of 200 mg endogenous Urea-nitrogen, of which 120 mg were excreted and 80 mg were used.

Rabbit "T" was dosed with 139 mg Urea orally three times daily and an antibacterial mixture of 35 mg sulfaquinoxaline plus 15 mg neomycin sulfate per day added to the Urea solution for a few days. The animal was deprived of drinking water during the experiment. Rabbit "T" excreted 521 and 161 mg Urea in

the urine and feces, respectively. Dosing with little water plus antibacterial agents increased the urine excretion of Urea to 717 mg and decreased the fecal excretion of Urea to 140 mg (Houpt 1963).

Schubert et al. (1991) orally dosed two lactating Shetland pony mares with Urea. The animals were dosed with concentrate (200 g/kg casein, 657 g/kg starch, 70 g/kg sugar, 30 g/kg sunflower oil, and 43 vitamins/minerals) and Urea at 6.00, 12.00, 18.00 and 24.00 h, straw was offered every hour and water was available ad libitum. Urea was not labeled on experimental days 1, 8, and 9 and was ^{15}N-labeled on days 2 to 7. During the accumulation period, the labeling of the nitrogen of the ration amounted to 13.35 and 15.34 atom% ^{15}N-excess (^{15}N′) for animals 1 and 2, respectively. The mares were sacrificed at 5.00 h on the 10th day of the experiment.

Animal 1 consumed 2400 g/day wheat straw, 1500 g/day concentrate, 20 g/day Urea (95% ^{15}N for day 6), 15000 g/day water and had a total nitrogen of 67.5. Animal 2 consumed 2000 g/day wheat straw, 1500 g/day concentrate, 20 g/day Urea, 17000 g/day water and had a total nitrogen of 59.2. Tissues with active metabolism had high markings (>0.3 atom-% ^{15}N′).

Eighty percent of the consumed ^{15}N′ was excreted in the urine and feces and about 2% was excreted in the milk as lysine, histidine, and arginine. Approximately 90% of the labeled nitrogen was recovered in each case. Blood plasma was labeled more quickly and achieved the plateau later than total blood. Urine labeling was more intensive and reached a plateau earlier than plasma and the ^{15}N incorporation into milk occurred at almost the same time as urine (just after the first dose at 6.00 h). The feces were labeled about 48 h later. The maximum ^{15}N′ secretion in milk was between days 4 and 6 of accumulation (Schubert et al. 1991).

Clinical Pharmacokinetics

Walser and Bodenlos (1959) injected normal healthy subjects IV with 25 to 100 mg [^{15}N]Urea or 2.5 μCi of [^{14}C]Urea. One glass of water per hour was ingested for 4 to 8 hours following administration of labeled Urea. Urinary catheters were not used to collect urine samples. Blood samples were obtained at intervals and immediately after the subjects voided. Plasma and urine samples were obtained at intervals of 1 to 3 hours and/or urine was collected for several days to determine the recovery of labeled material in the urine.

The first samples in experiment 1 were collected at least 90 min and up to 10 h after injection. The atoms percent excess ^{15}N or specific activity of ^{14}C in blood Urea had a linear decline on the logarithmic scale. However, some variations occurred with some of the subjects.

Representative data for labeled nitrogen in plasma and urine were provided for a healthy male, 74.1 kg, injected IV at time 0 with 100 mg Urea containing 60.9 atom% ^{15}N. The serum Urea nitrogen at time 0 h was 15.0 mg%. No significant changes were observed until 32 h, at which time serum Urea nitrogen had increased to 17.3 mg%. The level decreased to 14.1 mg% at 79 h and increased again to 17.4 mg% at 130 h. Urine Urea nitrogen steadily increased, although some deviations occurred. From 131 mg% at time 0, urine Urea nitrogen peaked between 32 and 56 h at 853 mg%. After 130 h the value decreased to 605 mg%. Urine ^{15}N was 0.415% between 0 and 2 h and steadily decreased until it was at 0% after 130 h. The percent cumulative dose for this individual increased from 19.7 to 79.5 over 130 + h. The urinary ammonia nitrogen and total urinary nitrogen both peaked between 32 and 56 h, then fluctuated until they had finally decreased by 130 + h.

The proportion of labeled and unlabeled molecules were slightly higher in the urine than in the blood in most of the intervals. The average urinary delay time ranged from 0.5 to 1.5 h for the first 8 hours following injection.

The investigators suggested that this unexpected delay time could be explained by the existence of a pool of Urea in the kidney parenchyma with which the excreted Urea is in equilibrium. The calculations for excess tracer excreted during the period of equilibrium indicated that 0% to 20% of the dose was reaching the kidneys during the various experiments. The investigators explained this by stating that during equilibrium, since Urea is highly diffusible, the labeled Urea may be localized for several minutes in the systemic organ it reaches on the first circulation. If that organ is the kidney, a large fraction will be excreted during equilibration; however, if it is another organ, only a small fraction may reach the kidney during equilibrium.

These authors calculated the Urea space as 56% to 70% of body weight. The Urea pool varied from 13 to 20 g and the rate of production and metabolism varied from 0.96 to 1.96 g/h and 0.15 to 0.43 g/h, respectively. The ratio of Urea metabolized to Urea produced between subjects receiving only [^{15}N]Urea or [^{14}C]Urea was not changed when tetracycline derivatives and/or sulfonamide and succinylsulfathiazole were administered. Subjects who received injections of [^{14}C]Urea only and [^{14}C]Urea plus neomycin (3 g/day for 2 days) had significantly increased recovery of labeled Urea (Walser and Bodenlos 1959).

Shannon and Prigmore (1961) orally dosed 11 subjects with 30 g of Urea; blood samples were collected at 20, 40, and 60 min after dosing. The serum Urea concentration mean prior to dosing was 36.4 ± 4.74 mg%, reached a maximum of 97.6 ± 9.17 mg% after 40 min, and remained high at 94.6 ± 8.72 mg% after 1 h. Another 10 subjects exposed to the same dose were studied over a 3-h period in which blood was taken at hourly intervals. The serum control mean was 36.1 ± 3.22 mg%, increased to 77.0 ± 9.78 mg% after 1 h, maintained this level at the 2-h time interval, and decreased to 72.7 ± 5.76 mg% at the 3-h interval. Twelve subjects dosed with 60 g Urea had blood samples collected for 1 h. The control serum Urea concentration was 29.7 ± 5.71 mg%, which increased throughout the 1-h period to a final level of 109.2 ± 23.6 mg%. Another 11 subjects received the same dose of Urea and were followed for a 3-h period. The control serum Urea concentration of 31.3 ± 5.58 mg%, peaked at 118.3 ± 10.37 mg% after 2 h, and dropped to 112.3 ± 10.94 mg% by 3 h.

Wolpert et al. (1971) studied Urea transport across the colon in eight male volunteers. The study was conducted using the following methods: control observations were made by perfusing the colon with an electrolyte solution; 16 to 20 g (containing 5 to 10 μCi of [^{14}C]Urea) of Urea were injected IV; 135 mg/min Urea was delivered to the colon (Urea is delivered to basal circulation at 38 mg/min) and the bacterial contribution to colonic urease activity was studied by treatment with 8 g/24 h neomycin for 3 days after IV administration of Urea. The Urea solution contained 9 g/L Urea and solutions were infused at 15 mg/min. Forty to 60 min after IV administration of Urea, blood Urea concentrations doubled and the concentration of Urea in the colon and colonic Urea output increased but Urea clearance did not change significantly. About 1.8% to 2.4% of Urea that reached the colon from the blood at normal or elevated blood Urea concentrations were recovered from the lumen. Urea absorption from perfusion of the solution through the colon was trivial and paralleled water absorption. During the next 48 h, 87% of the labeled Urea absorbed by the colon appeared in the urine. Most of the labeled Urea recovered from the rectum was unchanged, which confirmed that little Urea was exchanged between the colon and blood. After treatment with neomycin more Urea was recovered from the colon than before and the proportion of labeled Urea recovered from the urine during the next 48 h increased (Wolpert et al. 1971).

Yarnell et al. (1972) reported on seven patients who underwent Urea infusion therapy to reduce cerebral edema. The patients received intravenous infusions of 20.7 to 103.0 g/h, which yielded total doses of 0.59 to 1.21 g/kg. Two patients had 2-h infusions and five patients had 0.50- to 0.67-h infusions. Blood and urine were collected at regular intervals. One of the two patients that received 2-h infusions had plasma Urea concentrations that increased steadily for 2 h, peaked at 2 h (2.56 g/L) and then decreased slowly. Patients that received the 0.50- to 0.67-h infusions had plasma Urea concentrations that ranged from 1.58 to 3.40 g/L and peaked at 30 to 45 min. Six of the patients had average Urea clearance values that peaked at approximately 45 min.

Johnson et al. (1972) exposed three patients with advanced renal failure maintained by intermittent hemodialysis to Urea added to the dialysate to maintain blood concentrations of 181 to 600 mg/100 ml for 7 to 90 days. In all three patients, blood Urea concentrations $<$300 mg/100 ml were well tolerated, whereas concentrations above this were associated with malaise, vomiting, a tendency to bleed and headache. The more severe gastrointestinal, cardiovascular, mental and neurologic changes of uremia were not observed.

Picou and Phillips (1972) orally infused six well-nourished children (previously considered malnourished) receiving 3.7 g/kg/day protein with a milk mixture containing [^{15}N]Urea for 30 to 50 h. Dose was not available. Urea synthesis averaged 712 mg/kg/day and 65% was excreted in the urine. Of the unexcreted Urea, 61% or 156 mg/kg/day was used for protein synthesis, 27% was recycled, and 12% was excreted after further metabolism by the body. Reduction of the daily protein intake to 1.14 g/kg/day resulted in the average synthesis rate of Urea to decrease to 123 mg/kg/day and only 33% of this was excreted. Of the 91% unexcreted Urea, 45% was used for protein synthesis, 46% was used for other metabolic processes and 8% was recycled.

Varcoe et al. (1975) injected four normal subjects with a single intravenous injection of ^{125}I-labeled albumin and a combined injection of [^{14}C]Urea and [^{15}N]Urea. [^{14}C]Urea (58.1 mCi/mmol) was prepared as a solution of 10 μCi in 10 ml of 154 mmol/L sodium chloride and about 4, 8, or 16 mmol of [^{15}N]Urea was dissolved in the [^{14}C]Urea solution. At time zero, about 10 μCi of ^{125}I-labeled albumin was injected IV, immediately after a blood sample was taken, and 10 min later the combined [^{14}C]Urea and [^{15}N]Urea solution was injected IV. Blood samples were taken at 2-h intervals for 12 h and then daily for 10 days. Urine was collected at 2-h intervals for 12 h and then as pooled 24-h collections.

The mean values of the four subjects are presented in Table 5. The mean Urea half-life was $t_{1/2} = 8.3 \pm 0.9$ h. Two of the subjects were placed on a low-protein intake diet which increased their rate of Urea breakdown as a percentage of synthesis but not in absolute terms.

Albumin was detected in plasma as early as 2 h but some latent periods were as long as 3 to 4 h before [^{15}N]albumin was detected. The contribution of Urea nitrogen to the nitrogen required for albumin synthesis in normal subjects was small and protein restriction in two normal patients produced no effect on this relationship (Varcoe et al. 1975).

Gibson et al. (1976) obtained 29 ileostomy effluent specimens from 12 fit patients with normally functioning ileostomies. Bacteria in the specimens were inactivated by absolute ethanol and immediate cooling of the specimen. [^{14}C]Urea-turnover studies were carried out in six normal subjects and six subjects with nonfunctioning colons. The latter included four patients who had undergone colonic exclusion with ileorectal anastomosis and

TABLE 5
Urea metabolism data in four normal subjects (Varcoe et al. 1975)

Urea parameter measured	Value
Plasma Urea concentration	3.6 ± 0.3 mmol/L
Urea space	64.8 ± 2.4% body weight
Urea pool	158 ± 14 mmol/h
Urea clearance	2939 ± 170 ml/h
Urea synthetic rate	13.9 ± 1.6 mmol/h
Urea excreted	
mmol/h	10.7 ± 1.0
% synthetic rate	79 ± 5
Urea metabolized	
mmol/h	2.3 ± 0.5
% synthetic rate	17 ± 4

two patients with ileostomy. Control subjects of the [^{14}C]Urea-turnover study were on low- (40 g) and high- (100 g) protein diets. Subjects were stabilized on each diet for 7 days. The study started on the 8th day.

Of the six subjects with nonfunctioning colons, two were studied on the 40-g diet only with the protocol above and one was studied on both diets. Three subjects, who had recently undergone colonic exclusion, were studied on normal diets of about 70 g protein. The IV dose of [^{14}C]Urea for each experiment was about 5 μCi. Subjects were studied nonfasting. A steady state was confirmed by straight-line regression of log plasma [^{14}C]Urea radioactivity against time after at least 90 min had passed to allow equilibration of the injected tracer and by constancy of plasma Urea concentration. Immediately before iv injection of the tracer the urinary bladder was emptied. Timed blood and urine samples were collected at intervals of between 1 and 2 h over a 10-h period.

The ileostomy Urea concentration in the 29 specimens varied between 0.58 and 9.17 mmol/L. In 26 studies, the blood Urea was estimated at the time of ileostomy sampling and the ratio of ileostomy urea to blood Urea averaged 0.715 ± 0.40. The mean breakdown of Urea in the gut had a significant increase from 4.3 ± 0.49 to 7.6 ± 0.89 mmol/h from the low- to high-protein diet. The increased dietary protein intake also resulted in an increase in Urea production from a mean of 14.2 ± 1.3 to 28.2 ± 2.4 mmol/h.

The investigators concluded that, with normal renal function, a constant proportion of synthesized Urea is broken down in the gut. The volume of distribution decreased from 44.2 ± 2.1 to 43.3 ± 3.1 L from the high- to low-protein diet, whereas the blood Urea increased from 3.3 ± 0.2 to 5.9 ± 0.3 mmol/L. In control subjects, both the Urea pool and the excretion rate of Urea increased from the low- to high-protein diet, 142 ± 9.2 to 258 ± 2.8 mmol and 10.5 ± 1.0 to 20.5 ± 2.1 mmol/h, respectively. In patients with nonfunctioning colons, the gut breakdown as a percentage of production decreases significantly, from a mean of 28.3% in control subjects to 19.7% in patients with nonfunctioning colons. The volume of distribution, blood Urea, Urea pool, and excretion of Urea in nonfunctioning colons were 34.4 l, 4.3 mmol/l, 145 mmol, and 12.4 mmol/h, respectively (Gibson et al. 1976).

Gordon et al. (1976) reported a study in which eight normal volunteers received 5 to 6 μCi of [^{14}C]Urea orally or intravenously. The volunteers were allowed to sweat for 2 h in a hot, dry atmosphere while laying on a nylon mesh hammock suspended on a scale. Sweat solutes were eluted at the end of 2 h by washing the subject with distilled water. Weight loss during the heat exposure period was used to estimate original sweat volume. Subjects gave urine samples at the beginning and end of the sweating period. The period of sweat and urine collection began 90 min after the labeled Urea was given on day 0 and continued through the following 3 days. Two patients scheduled for amputation of an extremity received 100 μCi of [^{14}C]Urea 2 days prior to the operation. Three or more specimens of epidermis and underlying muscle were obtained as soon as possible after removal of the amputated limb.

The normal volunteers had more radioactive Urea in urine than in sweat on day zero. However, this relationship changed over the following 3 days and Urea in sweat became more highly labeled than Urea in urine. On days 0 to 3 the sweat:urine Urea ratio was 0.76 ± 0.04, 1.17 ± 0.07, 1.78 ± 0.17, and 3.54 ± 0.77, respectively. Two days after [^{14}C]Urea administration, patient 1 had skin and muscle samples with a ^{14}C content of 251 ± 12.6 and 124 ± 5.6 cpm/g tissue, respectively. Patient 2 had skin and muscle samples of 288 ± 22 and 20 ± 0.8 cpm/g tissue, respectively. Considerably more of the labeled Urea was stored in the epidermis than in muscle of these two patients (Gordon et al. 1976).

Saito et al. (1977) used radiolabeled Urea to study protein and nitrogen metabolism in normal and nephrotic patients. A normal subject, a chronic glomerulonephritic patient, and a nephrotic patient with azotemia were injected intravenously with 2 g of ^{15}N-labeled Urea of 50% purity, dissolved in 200 ml of physiological saline. The normal subject was on a normal diet, the chronic glomerulonephritic patient was on a moderate protein restriction of 30 g/day (2400 cal/day), and the nephrotic patient was placed on a 30 g/day (down from a 50 g/day) protein diet.

Dietary nitrogen and fecal nitrogen were not measured in the normal subject. The mean urinary nitrogen was 9.8 g/day in the normal subject, but less than half of that value for the chronic glomerulonephritic and nephrotic patients. ^{15}N incorporation into albumin contained in 100 ml sera was 0, 0.40, and 0.66 mg excess/dl for the normal subject, the chronic glomerulonephritic patient, and the nephritic patient, respectively. The authors suggest that the decreases in nitrogen excretion and the increase in nitrogen incorporation into albumin in nephrotic patients may relate to a decrease in amino acid degradation and corresponding increase in amino acid use for protein synthesis (Saito et al. 1977).

Long et al. (1978) performed Urea kinetic studies on five normal, healthy male and female volunteers (three females, two males, one male subject was used twice) and two septic patients (one male and one female). Four normal subjects received a nitrogen and calorie balanced diet 24 h before injection of ^{15}N- and ^{13}C-labeled Urea. Two of the normal subjects and the two septic patients received only infused glucose 24 h prior to and during the experiment. Urine was collected via catheter during 24 h of the study, except for two subjects who volunteered to void every hour. After emptying the bladder, a control blood sample was taken and 250 mg of pyrogen free highly ^{15}N- and ^{13}C-enriched labeled Urea were dissolved in 10 ml of saline and injected over a 2-min period. One subject received 500 mg neomycin six times daily to sterilize the bowel. Urine and blood were collected every 15 to 120 min for 24 h.

Urea nitrogen and total nitrogen were determined, and urine ammonia was isolated and quantified. A portion of the urine was reacted with urease and the ammonia liberated by reaction with saturated potassium carbonate was absorbed and analyzed to

determine the ^{15}N content in urinary Urea. The remaining samples were freeze dried, extracted with hot acetone, and repeatedly recrystallized to yield pure Urea. This material was reacted with hypobromite and potassium iodide and the nitrogen (N_2) gas produced was trapped, cooled and run through a mass spectrometer. If both nitrogens in the N_2 gas were ^{15}N isotopes, then the material was designated as mass 30 (15 + 15) Urea. If one nitrogen was ^{15}N and the other nonradioactive nitrogen, the material was designated as mass 29 (15 + 14) Urea. If both nitrogens were nonradioactive, the material was designated mass 28 Urea. The normal subjects injected with the ^{13}C- and ^{15}N-labeled Urea had body Urea composed of approximately 99.24% mass 28 Urea, 0.75% mass 29 Urea, and 0.005% mass 30 Urea.

In tracing the fate of the three Urea masses, the authors noted that the mass 30 Urea should diminish with time as Urea is metabolized. Mass 30 Urea metabolized in the body yields $^{15}NH_3$, which may be reused for Urea synthesis. New synthesis of mass 30 Urea is expected to be negligable so that new Urea produced from the nitrogen part of metabolized Urea would be mass 29 Urea.

In two subjects in which a 5-min sample was taken, maximum values for [^{15}N]Urea concentrations were observed. More than 100 min were required for uniform distribution in the body Urea pool. The Urea pool was calculated using six different methods; the average of the six methods indicated that subjects had a Urea pool size of 1983 to 6306 mg nitrogen.

A high correlation was observed between the cumulative percent dose excreted as Urea and the urine Urea ^{15}N or ^{13}C atom% excess in all subjects. Using this linear relationship, the authors calculated Urea pool sizes and found they were consistent with other methods of determining Urea pool size. The authors predicted there would be little ^{13}C reused from the labeled Urea, but the ratio of ^{15}N to ^{13}C in excreted urine was higher than predicted, suggesting recycling of carbon, possibly via a carbon intermediate common to the degradation and resynthesis pathways.

The average rate of Urea synthesis, excretion and metabolism was 24.6, 20.7, and 3.91 g/day, respectively. The average amount of nitrogen and carbon recycled was 2.56 and 2.53 g/day. For normal subjects on a reduced nitrogen diet or in the presence of an antibiotic, Urea breakdown was reduced. For the two septic patients in the study, the breakdown of Urea was negligible (Long et al. 1978).

Wrong et al. (1982) and Wrong and Vince (1984) reported on a study in which two healthy subjects were given a constant, three hourly diet to ensure a constant endogenous production of Urea. They were infused (IV) daily at a constant rate with 250 mg double-labeled ^{15}N-labeled Urea as a tracer. The mean ^{15}N enrichments found in plasma for each subject were 0.66 and 0.9 atoms excess percent. This procedure allowed them to maintain a constant ^{15}N enrichment of plasma Urea for 3 days, a period longer than that of a complete gastrointestinal transit. The ^{15}N enrichment of fecal ammonia was then compared with the plasma Urea-nitrogen that was circulating when the feces were formed in the body. Fecal ammonia nitrogen, whether obtained by in vivo dialysis, centrifugation of feces, or ultrafiltration of fecal incubate outside the body, had a ^{15}N enrichment that was 8.5% of the corresponding plasma Urea value. The enrichment of fecal total nitrogen was only slightly, but significantly, less at 6.8% of the plasma Urea value. Thus, over 90% of fecal ammonia nitrogen was derived from non-Urea sources, of which the major source may be protein in the intestinal bacteria.

Heine et al. (1984) administered a single oral tracer dose of 165 mg/kg [$^{15}N_2$]Urea to five infants fed breast milk, five infants fed a formula diet, and four infants given a bifidogenic formula diet. Infants were 1 to 11 months of age. An additional experiment was performed to test the effect of a continuously high Urea content on the intestinal flora. The stools were collected for 48 h and the stool bacteria were isolated. No significant differences with respect to ^{15}N excess of bacterial nitrogen and calculated ^{15}N incorporation as a percent of the administered dose was observed between the infants fed breast milk or formula. Infants fed a bifidogenic formula diet had significantly higher values for these parameters.

Moran and Jackson (1990a) injected 12 male subjects with 1.5 mg/kg [^{30}N]Urea ($^{15}N^{15}N$) through the biopsy channel of the colonscope. Six of the subjects were injected into the cecum and 6 were injected distal to the splenic flexure. The isotope was flushed through with 10 ml normal saline and the colonscope was removed without aspirating from the lumen. All urine and stools passed in the next 72 h were collected. Urine samples were collected from all subjects before injection of labeled Urea. Stools were passed 2 to 3 days after the procedure.

Most of the labeled Urea was excreted in the first 12 to 24 h. Peak enrichment occurred within 24 h and continued to decline to baseline during the 72 h period. The label was excreted as both ^{30}N- ($^{15}N_2$) and ^{29}N- ($^{15}N^{14}N$) Urea and was not statistically significant with respect to injections of the right or left colon. The median percentage of the dose excreted in the urine of the 12 subjects injected in the right and left colon was 6% and 4%, respectively. The recovery of labeled Urea was low; it varied from <4% to 12%. The retention of label from the right and left colon was 74% and 82%, respectively. No significant differences were observed for the excretion of labeled Urea between the right and left sides (Moran and Jackson 1990a).

Moran and Jackson (1990b) performed a study with a similar protocol as the previous study using [$^{15}N_2$]Urea or [^{30}N]Urea. Six subjects had two separate studies performed: the distal colon was intubated so that the tip of the catheter was just distal to the splenic flexure, the left defunctioned colon, and in the other study the proximal colon was intubated with the catheter tip in the middle third of the ascending colon, the right functioning colon. Five other subjects had the descending colon intubated proximally through the colostomy so that the catheter tip was in the region of the upper to middle third of the descending colon, the left functioning colon. The pilot study was performed on one patient in whose urine was collected every half-hour for 6 h and

in three aliquots thereafter for 48 h. The [^{30}N]Urea/[^{28}N]Urea and [^{29}N]Urea/[^{28}N]Urea ratios in the urine had a similar pattern of excretion. An early peak occurred at 1 h and was followed by a rapid decline which returned to baseline levels by 10 to 30 h. The findings indicated that at the 72-h urine collection one would expect to recover almost all of the label that would appear in the urine. Instillation of [^{30}N]Urea into the colon at colonoscopy resulted in 98% of the label recovered from the urine over 72 h in Urea.

Placement of [^{30}N]Urea in the defunctioned left colon resulted in a 29% median recovery in urine of labeled Urea over 72 h. Placement of the labeled Urea in the right functioning colon in the same subject resulted in a recovery of 9% of the dose. These results were statistically significant; however, recovery of labeled Urea from the right functioning colon of other subjects was not statistically significant but was statistically significant from the left defunctioned colon. Placement of [^{29}N]Urea in the colon did not significantly affect the percentage of the dose recovered in urine over 72 h from the left defunctioned colon or the right functioning colon.

Recovery for the left functioning colon was significantly greater than from the left defunctioned colon, but not significantly different from the right functioning colon. Labeled Urea placed in the right functioning colon was recovered within the total nitrogen of the stool by 36 h and represented a median of 7% of the dose. Two subjects had 29% and 37% of the label recovered when the isotope was placed in the functioning colon. The isotope was not recovered from the stool when placed in the left defunctioned colon. Most of the isotope was excreted in the urine as [^{29}N]Urea and [^{30}N]Urea and was excreted as ^{15}N in the stool. When the isotope was placed in the left defunctioned colon the median retention was 61% of the dose, which was not significantly different from 71% of the dose retained from the right functioning colon (Moran and Jackson 1990b).

Kalhan (1993) measured Urea and glucose kinetics in 33 infants during the first 3 days after birth. Nineteen of the infants had appropriate for gestational age (AGA) size, 11 of which were studied in the first 5 h after birth and 8 were studied after the feeding was started on the second or third day. Ten of the infants were infants of insulin-dependent diabetic mothers (IDMs), 6 of which were studied in the first 5 h after birth and 4 were started on the second day. Four of the infants studied were small for gestational age (SGA) and were studied between 8 and 36 h after birth. [$^{15}N_2$]Urea (99 atom% ^{15}N) and [6,6-$^{2}H_2$]glucose (98 atom% ^{2}H) were infused intravenously simultaneously. The bolus prime for [$^{15}N_2$]Urea was 2 mg/kg and the rate of constant infusion was 0.2 mg/kg/h. The bolus prime for [6,6-$^{2}H_2$]glucose was equal to 90 min of infusion or 2.7 mg/kg and the rate of constant infusion was 60 μg/kg/min. The tracers were infused for a 4- to 5-h period. Blood samples were drawn at 30-min intervals.

No significant effect on initiation of feeding was observed between the normal infants or IDM infants. The Urea nitrogen concentration was slightly lower in the SGA infants and higher in the IDM infants compared to the normal infants. Isotopic steady state was observed in all infants between 2 and 3 h. The rate of Urea synthesis in term AGA infants was 5.6 mg nitrogen/kg/h. Urea synthesis was slightly higher in infants studied after initiation of feeding. A higher rate of Urea synthesis occurred in the IDM infants and a lower rate occurred in the SGA infants; however, these differences were not significantly different from the normal infants (Kalhan 1993).

Odeh et al. (1993) conducted a study in which normal male (three) and female (two) subjects received intravenous injections of 4 g inulin and 2 g [$^{15}N_2$]Urea over a 5-min period. Blood samples were collected for 8 h after the injections and all urine voided during this period was collected. The administration of inulin and [$^{15}N_2$]Urea was well tolerated.

The subjects remained hemodynamically stable during the 8-h study period. Plasma versus time curves for Urea showed a three-compartmental structure of the kinetic model. The average distribution volumes for the five subjects were volume of central compartment (V_c) = 6.74 ± 0.91 L; volume of fast equilibrating peripheral compartment (V_f) = 10.88 ± 6.10 L, and volume of slow equilibrating peripheral compartment (V_s) = 30.04 ± 6.14 L.

In these five volunteers, the fast intercompartmental clearance (referenced to whole blood) was 2708 ± 267 ml/min; the slow intercompartmental clearance (referenced to whole blood) was 896 ± 148 ml/min; the renal clearance was 73 ± 10 ml/min; the nonrenal clearance was 16 ± 13 ml/min; and the Urea synthesis rate was 0.52 ± 0.15 g/day/kg. The permeability coefficient-surface area product of fast and slow compartments was 5110 ± 1520 and 1530 ± 470 ml/min, respectively (Odeh et al. 1993).

Pearson et al. (1994) treated six patients with end-stage renal disease (ESRD) (five female, one male) and five control subjects (three female, two male) with 100 mg of ^{13}C-, $^{15}N_2$-labeled Urea in 5 ml of saline as a 2-min intravenous infusion. Blood samples were taken before labeled Urea was given and at 2, 5, 10, 15, 30, 60, 120, 240, 360, and 480 min after infusion ended. The total clearance and volume of distribution (Vd) of ^{13}C-, $^{15}N_2$-labeled Urea in ESRD patients averaged 25.4 ± 11.5 ml/min and 28.6 ± 7.7 L, respectively. In controls the total clearance and Vd was 74.7 ± 38.5 ml/min and 43.3 ± 6.1 L, indicating a significant difference from ESRD. After Urea injection, the decay phase, which is attributed to the rapidly equilibrating compartment, falls quickly: $t_{1/2}$ is 6.4 ± 4.1 min. Only the terminal kinetic phase is visible after about 30 min. The investigators stated that both ESRD patients and controls yielded plasma concentrations that fit a two-compartment kinetic model.

Kaplan et al. (1999) gave four male and one female hemodialysis patients (ages 41 to 62 years and body weight range of 42.1 to 95.4 kg) 3 to 4 g of [$^{15}N_2$]Urea intravenously in one arm via timed 5-min infusions. These patients were stable, had been on maintenance hemodialysis for more than 1 year, and had plasma albumin greater than 4 g/dl. The normal volunteers were three males and two females ages 36 to 54 years. Blood was withdrawn

from the hemodialysis patients' contralateral arm at 5, 7, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 180, 300, 480, and 720 min for predialysis Urea kinetic analysis. At the time of the patients' regular dialysis session, arterial and venous samples were obtained at intervals of 15, 30, 60, 90, 120, 150, 180, 210, and 240 min for intradialytic Urea kinetics. Normal subjects received 2 g of [$^{15}N_2$]Urea IV over a 5-min period and blood was sampled from the contralateral arm at 5, 7, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 420, and 480 min. Urine samples were also collected from volunteers and patients throughout the study.

The mean steady state, central compartment (V1) and peripheral compartment (V2) volume of distribution (Vd) in predialysis patients was 45.9 ± 14.5 L, 18.0 ± 6.8 L, and 27.9 ± 9.5 L, respectively. Endogenous Urea production (EUP) was 22.6 ± 2.9 mg/min or 0.45 ± 0.14 g/kg/day.

Intercompartmental clearance between V1 and V2 averaged 1.26 ± 0.5 L. Significant renal clearance was not observed in any patients. The intradialysis Vd was 43.2 ± 14.5. Dialysis clearance was 216.6 ± 15.6 ml/min (predialysis was 5.9 ± 3.6 ml/min). Intercompartmental clearance and nonrenal clearance during intradialysis were not different from predialysis concentrations. Except for renal clearance, no statistical difference was noted between normal volunteers and patients in the parameters measured (Kaplan et al. 1999).

Skin Penetration

Treherne (1956) exposed whole skin and dermis from the abdominal region of rabbits in vitro to 0.01 to 0.05 M [^{14}C]Urea (1.0 mCi/mmole). The time delay for the penetration of whole skin after treatment with Urea was 64.3 ± 5.74 min. The permeability constant after treatment with Urea for whole skin and dermis was 2.37 ± 0.13 ($\times 10^6$) cm/min and 1.20 ± 0.09 ($\times 10^3$) cm/min, respectively. All values were the mean of four or five observations.

Franz (1978) determined the absorption of Urea through human abdominal skin obtained at autopsy. Although no dose was available, 5 to 10 μl of a liquid vehicle or 2 to 5 mg of a nonliquid vehicle was applied per square centimeter of skin. The median in vitro absorption of Urea was determined to be 11.1% of the applied dose from a total of 22 subjects. In in vivo studies, 4 μg/cm^2 of [^{14}C]Urea (1 μCi) dissolved in acetone was applied to the skin of the forearm. Total urinary output was assessed for 5 days. The mean absorption was $6.0\% \pm 1.9\%$ of the applied dose in four subjects.

In a study designed to compare in vivo and in vitro data, Bronaugh et al. (1982a) measured the percutaneous absorption of [^{14}C]Urea (53 mCi/mmol in petrolatum) in vivo in rats and in vitro in rat skin. The percutaneous absorption of benzoic acid, acetylsalicylic acid, and caffeine was also determined, but details were not given here. The quantity of [^{14}C]Urea per milligram of the petrolatum vehicle was 15 ng. In vivo absorption was determined from the radioactivity excreted in the urine over 5 days. In order to correct for the quantity of [^{14}C]Urea percutaneously absorbed but not recovered in the urine samples, 1 μCi of the compound was injected into the tail vein of separate animals and the percent recovery of radioactivity in the urine was determined. In vitro absorption was measured by standard diffusion cell techniques using full thickness, lightly shaved, skin with the subcutaneous fat removed. The receptor solution was saline containing 0.01% Thimerosol as a bacterial growth retardant.

In the in vitro experiment, 30 mg of petrolatum was applied using a spatula to a 1.13-cm^2 area of the mounted skin (to a calculated thickness of 0.2 mm). Samples were withdrawn periodically and counted in liquid scintillation fluid.

In the in vivo experiment, the rats were prepared by gently shaving the skin on the midback. A nylon ring with an inner area of 2.0 cm^2 was attached to the skin with a cyanoacrylate adhesive. Rubber tubing wrapped around the body of the rat, in front of the hind legs and behind the front legs, prevented the rat from scratching its back. The vehicle (50 mg) plus [^{14}C]Urea was applied to completely cover the skin inside the ring. A wire mesh covered the ring after dosing and the rats were housed in separate metabolism cages for collection of 24-h urine samples.

The total amount of [^{14}C]Urea eliminated was about 8% in the in vivo experiment and 5% for the in vitro experiment. The authors concluded that the quantitative agreement between the in vivo and in vitro experiments was good, and found that the rank order of penetration (qualitative agreement) of benzoic acid, acetylsalicylic acid, and Urea were the same for the two methods. The in vitro permeability constant for Urea was reported to be 1.6×10^{-5} cm/h (Bronaugh et al. 1982a).

In an extension of the work described above, Bronaugh et al. (1982b) compared in vitro absorption of benzoic acid, acetylsalicylic acid, and Urea in rat, hairless mice, miniature pig, and human skin. [^{14}C]Urea (53 mCi/mmol) was dissolved in 30 mg of petrolatum and applied to the stratum corneum of the skin of several species. The labeled Urea was spread into a smooth layer that completely covered a 1.13-cm^2 area of exposed skin. Samples (10 μl) were periodically withdrawn from the normal saline receptor solution to determine the steady-state rate of absorption up to 96 h. The approximate quantity of solute per milligram of petrolatum was 15 ng.

Skin was obtained from the backs of female Osborne-Mendel rats, female Swiss mice and female C3H mice from 10 to 20 weeks of age. The skin was used full thickness and had been gently shaved with an electric clipper and the subcutaneous fat removed. Skin from miniature Hormel strain pigs was sliced from the surface with a dermatome. This slice contained all of the epidermis and an upper portion of the dermis. The authors stated that this layer was similar in thickness to the distance traversed in vivo by an absorbed compound that has been taken up into the blood in the highly vascular region of the dermis. Human abdominal skin was obtained from both sexes at autopsy within 24 h of death. The epidermal layer was prepared by heat separation (60°C for 1 min). The integrity of each

sample of human skin was verified by measuring the permeability of ^3H_2O.

The rat and mouse skin were most permeable, with permeability constants that appear to be about 2×10^{-5} cm/h, whereas pig, human and hairless mouse all had permeability constants that appear to be $<1 \times 10^{-5}$ cm/h. Although all of the test compounds were ranked in the same order, independent of the skin type used, the authors recommended an initial comparison to human skin before selecting an animal skin as a surrogate. For example, they cited the benefit of testing a slowly penetrating chemical such as Urea in an animal with few hair follicles so that the results are not unnaturally skewed by greater diffusion through the region of the follicle (Bronaugh et al. 1982b).

Bronaugh et al. (1983) studied the differences in in vitro skin permeability as a function of skin site and sex of the animal. Using the same techniques as described above, approximately 854 ng of [^{14}C]Urea (53 mCi/mmol in petrolatum) was applied to skin harvested from the back or abdomen from male and female Osborne-Mendel rats. The permeability constants were $1.6 \pm 0.5\ (\times 10^{-4})$ cm/h and $18.8 \pm 5.5\ (\times 10^{-4})$ cm/h for the back and abdominal skin of male rats, respectively. The permeability constant was $4.8 \pm 1.3\ (\times 10^{-4})$ cm/hr for the back skin of female rats. The lag times for back and abdominal skin for male rats were 15.0 ± 1.8 and 16.5 ± 4.3 h, respectively. The lag time for female rats was 11.1 ± 0.6 h. The authors concluded that there are differences in absorption as a function of site and sex of the animal. In an attempt to explain the differences, the authors measured stratum corneum, epidermis, and whole-skin thicknesses as a function of site and sex, and included data on castrated males. The skin thickness measurements of castrated males are similar to those of females. Urea permeability constant, however, was not given for the castrated male skin.

Bronaugh and Stewart (1985) performed in vitro studies using intact and abraded human abdominal skin and dorsal skin from female Osborne-Mendel rats. The area of skin exposed to [^{14}C]Urea (57.0 mCi/mmol) in a cosmetic lotion (5 mg/cm^2) for 24 h was 0.32 cm^2. The site of application was washed with soap and water at 24 h to remove unabsorbed material. The absorption of Urea across normal and abraded human skin was $9.5\% \pm 2.3\%$ and $67.9\% \pm 5.6\%$, respectively. This represented a 7.1-fold increase and was based on four determinations. The absorption of Urea across normal and abraded rat skin was $11.5\% \pm 3.1\%$ and $58.7\% \pm 7.2\%$, respectively. This represented a 5.1-fold increase and was based on 6 determinations.

Petersen et al. (1986) grafted human skin obtained from females undergoing abdominoplasty on to athymic nude mice (11 to 16 weeks old). The human skin was obtained after surgery and was about 0.4 mm thick. The mouse's skin behind the left front leg was clipped away and human skin was placed over the denuded area. Vascularization took 3 to 5 days and healing was complete in about 3 to 4 weeks. The human skin graft was outlined by a template and test and control preparations were administered uniformly over the marked area of 1 cm^2. Animals were restrained in a standing position by placing the feet through holes in a plastic device. ^{14}C-labeled Urea (5 μl) with a specific activity of 7 μCi/ml and a concentration of 0.1% at pH 7.2 was applied to two mice. A recovery time of 4 h was allowed. After the applied dose had dried, the external skin surface was cleansed with water and cotton swabs. Twenty tape strippings were performed to remove both mouse and human stratum corneum.

Microscopic examination of mouse skin showed that the mouse stratum corneum had 7 to 12 layers and human stratum corneum had 12 to 23 layers. The investigators suggested that a possible thickening of the corneum may have occurred during transplantation. The percent of applied radioactivity recovered in the 20 strips was as follows: human transplant (left front leg), 17.13%; mouse skin, right front, 14.76%; mouse skin, left back, 16.54%; and mouse skin, right back, 15.41%. A high stratum corneum uptake of Urea occurred (Petersen et al. 1986).

Ackermann and Flynn (1987) determined the permeability coefficients for [^{14}C]Urea as an ethanol solution in male nude mice older than 90 days. The epidermis was harvested from full-thickness abdominal hairless mouse skin. The concentration of [^{14}C]Urea in the donor cell was 9.2×10^{-6} M. Unlabelled compound was also placed in the receiver and donor cells to facilitate permeation of the labeled compound. However, because of the slow build-up of radiolabeled compound in the receiver cell it was difficult to detect the concentration of compound in the receiver cell. As a result, inaccurate data were obtained over the first 6 to 10 h of the experiment. Therefore, the permeation profile was determined by measuring loss of [^{14}C]Urea from the donor cell. The permeability coefficients for full-thickness skin and dermis were 1.2×10^{-4} cm/h and 0.68 cm/h, respectively. A continuously increasing permeation rate as a function of time occurred. The investigators suggest that the percutaneous absorption of Urea takes place through hydrophillic and water-filled regions in the skin.

Auclair et al. (1991) conducted a study of Urea absorption in rats. Hairless Sprague-Dawley, SPF rats were prepared with a bipediculated dorsal flap (BDF). One day prior to the experiment a catheter was introduced into the jugular vein. [^{14}C]Urea with a specific activity of 2.07 Gbq/mM and a concentration of 0.21 mg/ml in an ethanolic solution was used in the study. The [^{14}C]Urea solution (25 μl) was applied to each flap pedicure during a 4-h period on two circular areas 17 mm in diameter. A ligature was put at the root of the left pedicle just before application. Changes in cutaneous blood flow after ligating one pedicle were measured by an iv injection of an isotonic aqueous solution of ^{201}T (37 Mbq/ml) 15 min before sacrifice. The stratum corneum was removed by about 15 successive strippings using adhesive tape on each treated area of the flap. Urea was applied to the stripped skin, and the animals were killed 30 min later. No further stripping was performed.

The decrease of cutaneous blood flow after applying Urea was assessed by measurement of ^{201}T distribution from free to ligated pedicle. These experiments were reproducible with a mean decrease of $65\% \pm 15\%$. After a 30-min application of

Urea on stripped skin, the percentage of blood flow decrease was $65\% \pm 16\%$. The mean quantities of Urea obtained in the BDF skin were 0.12 ± 0.01 nM (superficial dermis) and 0.08 ± 0.01 nM (deep dermis). In the ligated pedicle the mean amount of Urea found in the superficial and deep dermis was 0.17 ± 0.04 nM and 0.10 ± 0.02 nM, respectively. No significant differences in the concentration of Urea in either the superficial or deep dermis were found between the two pedicles. The 12 rats that were dosed on normal skin (20 to 400 μm) had 0.12 ± 0.01 and 0.17 ± 0.04 nM Urea in the superficial dermis of ligated and nonligated pedicles, respectively. These same rats had 0.08 ± 0.01 and 0.10 ± 0.02 nM Urea in the deep dermis (400 to 920 μm) of ligated and nonligated pedicles, respectively. Six additional rats received similar doses of [^{14}C]Urea on stripped skin. The ligated and nonligated superficial dermis of these rats had 0.92 ± 0.44 nM ($p < .05$) and 0.27 ± 0.28 nM Urea, respectively. The ligated and nonligated deep dermis of these rats had 0.68 ± 0.53 nM ($p < .05$) and 0.13 ± 0.09 nM Urea, respectively. The increases in ^{14}C-Urea in the ligated versus nonligated pedicle were 240% in the superficial dermis and 423% in the deep dermis (Auclair et al. 1991).

Barber et al. (1992) studied the in vitro percutaneous absorption of [^{14}C]Urea in human and male Sprague-Dawley rat skin. Cadaver specimens did not have any adverse clinical skin condition or disease and skin samples were stored appropriately. Cadaver skin specimens were harvested 2 to 24 h postmortem from black and white males and females. Skin was surgically removed from the shaved abdominal area of euthanized rats. Skin samples were either human stratum corneum or full-thickness rat skin. The day of the experiment, the receptor chamber was filled with the receptor fluid, Dulbecco's phosphate-buffered isotonic saline (pH 7.1), containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Urea was tested as a dilute (53 μg/ml) aqueous solution. Background samples were taken from the receptor chamber of each cell at 0 h and then 300 μl of [^{14}C]Urea was applied in the donor chamber. The diffusion cells were incubated at a constant temperature of 37°C or 30°C for 8 h. The permeability of human and rat skin to tritiated water was evaluated before and after exposure to [^{14}C]Urea. Duplicate studies were performed for most experiments.

The first 1 to 2 h of the study were characterized as an initial lag phase followed by linear, steady-state absorption kinetics for the remainder of the study. The respective absorption rate and permeability constant for Urea in full-thickness rat skin were 1.81 ± 0.71 ($\times 10^{-5}$) mg/cm^2/h and 3.41 ± 1.34 ($\times 10^{-4}$) cm/h ($n = 6$). The respective absorption rate and permeability constant for Urea in human stratum corneum skin were 7.82 ± 3.39 ($\times 10^{-6}$) mg/cm^2/h and 1.48 ± 0.64 ($\times 10^{-4}$) cm/h ($n = 6$). The respective absorption rate and permeability constant for ^3H_2O in full-thickness rat skin were 2.97 ± 1.26 mg/cm^2/h and 2.97 ± 1.26 ($\times 10^{-3}$) cm/h. The respective absorption rate and permeability constant for ^3H_2O in human stratum corneum were 1.56 ± 0.83 mg/cm^2/h and 1.56 ± 0.83 ($\times 10^{-3}$) cm/h. The rat/human ratio of ^3H_2O to Urea was 1.90 and 2.31, which indicated that these compounds penetrate full-thickness rat skin more rapidly than human stratum corneum. The mean damage ratios (the ratio of the permeability constants for ^3H_2O after contact to that determined before chemical exposure) were 1.27 ± 0.15 and 1.42 ± 1.10 for full-thickness rat skin and human stratum corneum, respectively. The saline control values were 1.36 ± 0.45 and 1.60 ± 0.82 for full-thickness rat skin and human stratum corneum, respectively (Barber et al. 1992).

Lodén et al. (1995) studied 10 healthy volunteers with no visible signs of skin diseases in a double-blind study. Urea was dissolved in an aqueous vehicle containing 30% ethyl alcohol. Fifty microliters of a 2.5% or 10% Urea solution was pipetted onto one layer of filter paper placed in a large aluminum Finn Chamber. The chambers were placed on the inner upper arm of the volunteers and removed 3 to 6 h later. The test area was then stripped six times with transparent tape and measured with a digital light measuring instrument. In an in vitro study, human breast skin was mounted in diffusion cells of stainless steel and solutions of ^{14}C-labeled Urea (200 μl/cm^2) were applied to the outer surface. Three and 6 h later the skin was stripped six times with adhesive tape. The amount of Urea in each strip was analyzed in a liquid scintillation counter.

As the number of strippings increased, the amount of skin material decreased, as confirmed by absorbance through the strips. No significant difference was observed for the amount of skin adhering to the tape between the Urea and vehicle treated sites. The amount of Urea on the tape strips decreased slightly with the number of strippings, but was not as pronounced as the decrease in skin material adhering to the tape strips. The amount of ^{14}C-labeled Urea found on the tape strips was between 3 and 15 μg/cm^2. Larger amounts were found after exposure to the higher concentration of Urea (Lodén et al. 1995).

Michel et al. (1995) used human keratinocytes and dermal fibroblasts obtained from normal adult skin to determine Urea absorption. Hairless mouse abdominal skin, without fat, was also used in this study. Human keratinocytes were seeded at 2×10^5/cm^2 on an anchored dermal equivalent to produce an anchored skin equivalent (ASE) that was raised at the air-liquid interface 24 h after confluence. The samples were used 20 days later to determine percutaneous absorption by exposure to [^{14}C]Urea (7.8 mCi/mmol) and to perform lipid analysis. The labeled Urea (100 μl) was diluted in 0.9% NaCl and placed in the donor compartment. The radioactivity in the dermal bathing solution was evaluated at 1, 2, 4, 6, 8, and 12 h. A lag-time of about 8 h occurred for the penetration of [^{14}C]Urea through the ASE. However, the compound was completely absorbed after 24 h. The mouse skin had an increased lag-time and only 60% was absorbed after 24 h. Normal human skin had a short lag-time; however, <5% was absorbed after 24 h.

Lipid analysis revealed that human epidermis contained a total lipid concentration of 1 mg/cm^2 which was three to seven times greater than mouse and ASE epidermis. ASE epidermis had a qualitative lipid profile similar to human and mouse skin. Although ceramides, cholesterol, cholesterol sulfate, and

phospholipids were present in ASE, the proportions differed from normal human skin. The comparable qualitative results reflected the similar types of lipids present in the three systems, while the quantitative differences observed in the rate of absorption of organic compounds correlate with the lower amount of lipids (Michel et al. 1995).

Ogiso et al. (1995) studied the influence of other agents on Urea absorption. Skin samples were excised from the abdominal area of male hairless rats. The dermal side of the skin was soaked in a buffer solution for 12 h to equilibrate the skin. The skin was mounted in a Franz diffusion cell and a 0.5% Urea–55% ethanol solution with or without enhancer at the concentration indicated was placed in the donor compartment and occluded with a sheet of film. The receptor compartment was filled with 0.85% NaCl–10 mM phosphate buffer with a gentamicin solution in the ratio 1:100. Aliquots of 50 μl were withdrawn periodically up to 36 h.

Urea penetration was greatly enhanced by laurocapram (2% and 5%), *n*-octanol (20%), sodium oleate (1% and 3%), oleic acid (5%), monoolein (5% and 10%), and cineol (2% and 5%). Whereas cineol, 1% sodium oleate, and 2% laurocapram had high enhancing effects on the penetration of Urea, *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), and oleyl oleate produced little increase in the penetration rate of Urea (Ogiso et al. 1995).

Peck et al. (1995) measured the permeability coefficient of [^{14}C]Urea in human skin at temperatures that varied from 25°C to 45°C at 5°C intervals and then returned to 25°C to check for irreversible alterations in the human epidermal membrane (HEM) permeability. Samples were withdrawn from the receiver at 4-h intervals after allowing 12 h for the permeation rate to reach pseudo-steady-state conditions. Following this permeation experiment at 25°C, the temperature was increased by 5°C and the protocol was repeated. A similar procedure was followed for permeability coefficients at 27°C and 39°C. The test for irreversible changes in the HEM indicated that the permeability increased by an average of 25% for the four HEM samples exposed to temperatures that ranged from 25°C to 45°C. However, due to the irreversible changes in the HEM that would lead to significantly increased values at this temperature range, the procedure was changed to a two temperature protocol. The permeability coefficient at 27°C and 39°C was 2.6×10^8 and 4.1×10^8 cm/s, respectively.

Peck et al. (1998), simultaneously measured [^{14}C]Urea and [^{3}H] sucrose across HEM samples under passive and 250-mV conditions. The flux measurements were made before, during, and after HEM exposure to sodium dodecyl sulfate (SDS). The solutions were 0.1 M ionic strength phosphate-buffered saline (PBS). The first phase of the study involved an initial passive permeability phase and a 250-mV phase to assess the baseline properties of the HEM sample before surfactant was added. The cell was then rinsed with SDS. Stage 2 was successive measurements of passive and 250-mV iontophoretic permeability with 1 mM SDS in the diffusion cell after an 18-h SDS pretreatment phase. The diffusion cell was then rinsed with PBS. Stage 3 involved the measurement of passive and 250-mV iontophoretic permeabilities without surfactant in the diffusion cell after about 24 h in PBS. The experiments were repeated four times.

The average permeability coefficient ($\times 10^8$ cm/s) for Urea during passive and 250 mV iontophoresis during stage I was 7.7 ± 3.5 and 12 ± 6, respectively. The average permeability coefficient ($\times 10^8$ cm/s) for passive and 250-mV iontophoresis during stage 2 was 230 ± 130 and 640 ± 290, respectively. The average permeability coefficient ($\times 10^8$ cm/s) for passive and 250-mV iontophoresis during stage 3 was 104 ± 47 and 209 ± 85, respectively. The surfactant increased Urea permeability by a factor ranging from 14 to 42. The average pore size remained constant from stages 1 to 3. The investigators suggested that the observed increase in the passive permeability of HEM with SDS treatment was caused by the formation of small defects in the lipid and/or protein domains of the stratum corneum. Additionally, direct evidence from the studies which applied voltage to the HEM/Urea/SDS system supported the idea that the mechanism for flux enhancement within each stage of the protocol was electroosmosis. The Peclet number (*Pe*) was defined as the value directly proportional to solvent flow velocity. This value was largest during stage 2 for Urea, indicating that SDS enhanced the contribution of electroosmosis to the overall flux during this stage. The *Pe* value for stage 3 was lower than stage 2, but not as low as the baseline values estimated from stage 1 (Peck et al. 1998).

Penetration Enhancement

Wahlberg and Swanbeck (1973) used human and guinea pig skin to study the effect of Urea on the percutaneous absorption of hydrocortisone. Human skin from mammary plastic surgery and excised skin from the back of guinea pigs were used. The vehicles for hydrocortisone were: cream base with 10% Urea (pH = 3); cream base without Urea (pH = 3); 2.5% Tween 20 + distilled water; 2.5% Tween 20 + 10% Urea + distilled water; 2.5% Tween 20 + 10% Urea + 5% lactic acid + distilled water; and 2.5% Tween 20 + 5% lactic acid + distilled water. The vehicles for Urea were: distilled water; 2.5% Tween 20 + distilled water; and 2.5% Tween 20 + 5% lactic acid + distilled water. [^{14}C]Urea and [^{14}C]hydrocortisone were applied to the epidermal side (area 3.1 cm^2) of the in vitro apparatus, while the dermal side was bathed continuously in saline.

Hydrocortisone absorption over 72 h increased through human skin with the addition of 10% Urea (pH = 6.8), although it was not significant, whereas absorption through guinea pig skin decreased compared to controls. The addition of 5% lactic acid to the 10% Urea formulations gave the highest absorption for guinea pig skin. At 0 to 24 h the percent of applied hydrocortisone (0.03) that was absorbed versus control was 0.29 ± 0.09 and 0.12 ± 0.05, respectively. By 49 to 72 h, the percent absorption of the amount applied versus control was 0.94 ± 0.25 and 0.70 ± 0.23, respectively. Human skin had a higher absorption with the addition of 5% lactic acid to the 10% Urea formulations than the control or Urea only formulation.

Three experiments with a 1% hydrocortisone application (0.387, 0.0695, and 1.074 g) without Urea on guinea pig skin revealed no absorption in one experiment, 0.30% absorption by day 5, and 0.30% absorption by day 4 with no hydrocortisone detectable on day 5. Experiments with this same hydrocortisone cream in a 10% Urea formulation (0.780 and 0.969 g applied) reported 0.06% absorption by day 5 with the lower dose and no detectable absorption with the higher dose.

The percent Urea absorbed from human skin exposed to a 10% Urea concentration in distilled water (2.0 ml) ranged from 0.02% to 0.13% over a 7-day period, whereas that from guinea pig skin ranged from 0.01% to 2.48% over the same 7-day period. A 1.0 ml application of 20% Urea concentration +2.5% Tween 20 + distilled water to guinea pig skin resulted in a 0% to 1.99% absorption of Urea. This same formulation plus 5.0% lactic acid resulted in the absorption of 0.04% to 4.02% Urea; however, in one experiment no Urea was detected on days 4 to 7. In human skin, this same formulation resulted in a 0% to 2.02% absorption of Urea over a 7 day period. The absorption of Urea from human and guinea pig skin was not linear (Wahlberg and Swanbeck 1973).

Ohshima et al. (1984) used male Wistar rats to study the effects of Urea on the percutaneous absorption of 6-carboxyfluorscein (CF). One day prior to the experiment the hair of the abdomen was carefully shaved. A glass chamber (inside diameter = 3.2 cm) was applied to the shaved abdomen using cryoacrylate adhesive. Blood samples were collected at 1, 2, 3, 4, 5, and 6 h after 10 ml of 0.02% *w/v* CF was placed in the glass chamber as the control experiment. The same concentration of CF with the absorption promoters 10% *w/v* Urea and 0.4% *v/v* 2-mercaptoethanol (MER)–9.6% *w/v* Urea (coadministered as a protein solubilizer) were placed in glass chambers. The stratum corneum was removed slowly twice by cellophane tape and was confirmed microscopically. The glass chamber was then attached to the skin. A 10-ml specimen of each reagent was placed in the glass chamber. Blood samples were collected as described in the control experiment.

In the control experiment, the plasma CF concentrations reached a plateau after 1 h. At 6 h post dosing, the plasma CF concentration was less than 3 ng/ml. The 10% *w/v* Urea solution resulted in a constant plasma CF concentration at least 1 h after percutaneous administration. The plasma CF concentration at 6 h after dosing was 2.5 times higher than the control experiment. Plasma concentrations gradually increased up to 6 h after administration of the 0.4% *v/v* MER–9.6% *w/v* Urea. At 6 h after topical administration of 0.4% *v/v* MER–9.6% *w/v* Urea plasma CF concentrations increased to 11 ng/ml. Plasma CF concentrations 6 h after administration were increased 60 times by mechanical removal of the stratum corneum compared to non-stripped control experiments (Ohshima et al. 1984).

Ishikura et al. (1987) deposited various chemicals on plastic film and applied the film to rabbit skin. Diltiazem hydrochloride (DIL) was dissolved in 40 ml of 80% ethanol and 0.6 g of carboxyvinyl polymer (CVP), 0.6 g of hydroxypropylcellulose (HPC-H, -L) and 0.1 g of Urea were added and the mixture was homogenized. The homogenate was spread over a surface area of approximately 16 × 16-cm of Saran Wrap and dried for 15 h at 45°C to 55°C. The Saran Wrap was cut into four sheets of 7 × 7 cm area containing 90 to 100 mg of DIL each. Four films containing 70 to 80 mg of disodium cromoglycate (DSCG) were prepared similarly from an aqueous mixture of 0.6 g of CVP, 0.6 g of HPC-L, 0.51 g of DSCG, and 0.09 g of Urea. These procedures are referred to as method A. Method B used electrically neutral bases and film preparation that consisted of mixing 0.6 g DIL, 0.6 g glycerol, and 0.10 g of Urea added to a low-viscous aqueous solution of 0.6 g of polyvinylalcohol (PVA). DSCG films were prepared in an aqueous mixture of 0.6 g PVA, 0.51 g of DSCG, 0.6 g of glycerol, and 5% *w/w* of Urea. Films were obtained in a similar manner to method A.

The films were applied to the abdominal region of albino male rabbits. The hair had been removed with an electric clipper followed by hair-removing cream the day before application. The rabbits bodies were wrapped tightly after the film had been applied. Intravenous studies were also performed. Aqueous solutions of DIL and DSCG were injected into the external auricular vein at doses of 2 mg/kg and 10 mg/kg, respectively. Blood samples were collected at 1, 2, 4, 7, and 24 h after application of the film and 3, 5, 15, 30, 60, 90, 120, 180, and 240 min after IV administration. The stratum corneum of the skin was removed by serial stripping with adhesive tape on the shaved abdominal region.

These authors reported the bioavailability of DIL over 24 h for the control (no additive) as 39% and 0% to 5% for stripped and intact skin, respectively ($n = 1$). The bioavailability for DIL when treated with 5% Urea was 42% for stripped skin and 2% for intact skin; 38% for stripped skin and 4% for intact skin treated with10% Urea; and was 45% for stripped skin and 6% for intact skin treated with 20% Urea.

The bioavailability of DSCG over 24 h for the control (no additive) was 105% and 0% for stripped and intact skin, respectively ($n = 1$). The bioavailability for DSCG when treated with 5% Urea was 110% for stripped skin and 0% for intact skin; 130% for stripped skin and 1% for intact skin treated with 10% Urea; and 50% for stripped skin and 13% for intact skin treated with 20% Urea. The bioavailabilities of DIL and DSCG over 24 h were about 40% and 100%, respectively, of the values obtained for IV administration, whether the films contained additive or not. These values were reported using method A.

The plasma bioavailability of DIL 1 h after application to the stripped skin using method B was 117%, whereas the application of DIL to intact skin resulted in almost no absorption (2% ± 1%). Values were not given for DIL plus Urea applied to stripped skin using method B. The bioavailability for DIL plus Urea ($n = 3$) applied to intact rabbit skin was 22% ± 10%. The bioavailability of DSCG applied to intact rabbit skin was 0%, whereas the addition of Urea increased this value to 32% ± 5%. The application of Urea plus anionic surfactants, amphoteric surfactants or dehydrocholic acid to intact rabbit skin using method B ($n = 3$)

increased the bioavailability of DIL over the control value (Urea alone). The application of Urea plus tetra-*n*-butylammonium bromide and choline chloride to intact rabbit skin using method B ($n = 3$) increased the bioavailability of DIL over the control value (Urea alone) (Ishikura et al. 1987).

Lata et al. (1988) used a Urea pretreatment in a study of the effectiveness of β-aminopropionitrile (β-APN) fumarate, a collagen cross-linking inhibitor. Twenty female albino Wistar rats (5/group) were anesthetized and a 2.5-cm^2 area of skin was closely shaved and implanted subcutaneously (SC) with two polyvinylalcohol (PVA) sponges. The control group had 30% DMSO applied on the intact skin over the PVA sponge placement. In the three experimental groups β-APN was prepared in 30% DMSO and 0.1 ml was applied to 2.5 cm^2 once daily for 8 days. β-APN was applied to the skin three different ways: (1) painted directly on the skin; (2) gauze was soaked in the same volume of β-APN and the application site occluded with an impermeable membrane, and (3) the shaved skin was pretreated with 20% Urea for 5 min, followed by cleansing of the skin and application of the gauze soaked with β-APN and then occluded.

The total content of granuloma tissue components, such as DNA, noncollagenous proteins, and collagen were not affected by the three β-APN methods of administration onto intact skin. The effectiveness of β-APN was significantly enhanced by pretreatment of skin with Urea. The solubility of insoluble collagen in denaturing agents was significantly greater in 6 M Urea for the β-APN + Urea + occlusion versus the control group and the groups painted with β-APN with and without occlusion. A significant increase in the extractability of collagens into neutral salt was observed in all β-APN–treated groups compared to controls. A significant decrease in body weight gain and granuloma tissue (gram/pellet) was observed in rats treated with β-APN + Urea + occlusion (Lata et al. 1988).

Nishihata et al. (1990) investigated the in vitro transport of indomethacin across excised rat dorsal skin (four samples per treatment), with and without the addition of 5.0 g Urea and 5.0 g of ethanol, butanol, octanol, decanol, dodecanol, or tetradecanol. The exposed area of skin was 5.3 cm^2 and the weight of the aqueous gel was 0.5 g, which contained 500 μg of indomethacin.

The addition of Urea (plus no alkanol) increased the transport of indomethacin but it was not a statistically significant increase. The percent of indomethacin transported after 12 h increased with and without Urea when the alkanols ethanol, butanol, octanol, or decanol were added to the aqueous gel, although gels with Urea had greater percentage of indomethacin transport than those without Urea. Gels that contained dodecanol or tetradecanol with and without Urea had a decreasing percent transport of indomethacin after 12 h relative to the other alkanols. Again, the values were greater for the gels that contained Urea.

The increased transport of indomethacin was considered significant when Urea plus 1-octanol, 1-decanol, or 1-dodecanol were present in the gel. Transport rates of indomethacin in the presence of Urea were two times greater than in the absence of Urea. Additionally, the transport of indomethacin was about two times greater in the presence of 1-octanol, 1-decanol, or 1-dodecanol. Additional data from Nishihata et al. (1990) are presented in Table 6.

TABLE 6

Transport of indomethacin across rat dorsal skin in aqueous ointment formulations (Nishihata et al. 1990)

Constituents of aqueous gels[a]	Transport at steady state (μg/h)	Urea/no Urea transport ratio at steady state	Percent indomethacin transported after 12 h	No. of samples
2.5 ml of 500 μg indomethacin	1.62 ± 0.59	—	3.2 ± 1.1	4
no alkanol, no Urea	6.52 ± 0.79		11.6 ± 1.6	4
no alkanol, 5.0 g Urea	8.57 ± 1.24	1.31	14.7 ± 3.4	4
5 g ethanol, no Urea	6.46 ± 1.46		11.3 ± 2.1	3
5 g ethanol, 5.0 g Urea	8.69 ± 2.09	1.34	15.0 ± 2.5	3
5 g butanol, no Urea	6.74 ± 1.18		13.4 ± 4.2	3
5 g butanol, 5.0 g Urea	8.91 ± 1.41	1.32	15.8 ± 2.1	3
5 g octanol, no Urea	10.04 ± 1.16^b		19.9 ± 3.4^b	4
5 g octanol, 5.0 g Urea	19.97 ± 1.52^c	1.99	34.1 ± 4.2^c	4
5 g decanol, no Urea	11.56 ± 1.61^b		23.6 ± 2.7^b	4
5 g decanol, 5.0 g Urea	22.39 ± 1.94^c	1.94	41.9 ± 5.7^c	4
5 g dodecanol, no Urea	9.81 ± 1.04^b		20.3 ± 3.5^b	4
5 g dodecanol, 5.0 g Urea	18.21 ± 1.62^c	1.86	36.6 ± 6.1^c	4
5 g tetradecanol, no Urea	8.12 ± 2.55		14.2 ± 3.9	3
5 g tetradecanol, 5.0 g Urea	10.66 ± 2.97	1.28	24.1 ± 3.7^c	3

[a] Included 100 mg indomethacin, 6.5 g phospholipid, 6.5 g Witepsol H-15, 10 g polyethyleneglycol 400, and 66.9 to 76.9 g 0.1 M sodium phosphate buffer at pH 7.2.

[b] $p < 0.01$ versus no alkanol/no Urea group.

[c] $p < 0.01$ versus no alkanol/no Urea group and no Urea group.

Rahman et al. (1990) studied the in vitro release of naproxen from two bases, a water-washable base with the drug in the water phase and a hydrophillic base with the drug in the water phase, plus 1%, 5%, and 10% Urea. Experimental data reported that more naproxen was released when incorporated into the water phase of the formulations. Samples of the diffusion medium were withdrawn at 5, 15, 30, 45, 60, 90, and 120 min. At 1% and 5% concentrations, Urea increased the availability of naproxen from the two bases; however, at a 10% Urea concentration the release of the drug was decreased.

Han et al. (1991) exposed rabbits to 10% salicylic acid or 11.6% sodium salicylate and 10% Urea. The ointment base was white vaseline. Hair was removed from the skin of the dorsal area on both sides of the spine and 6.9 g of the ointment was uniformly spread over an 8 × 10-cm shaved area of the animal. The treatment area was covered with a linear-low-density polyethylene wrap film which was wrapped with adhesive bandage. The rabbits were immobilized during the experiment. The ointment was left on the skin for 6 h and food and water were withheld during that time.

The application was removed and the treated area was thoroughly washed with warm water on completion of a single test. The ointment left on the application site and the washings were collected. A 7-day rest period ensued before reapplication of ointment. Blood was withdrawn from the marginal ear vein 30 min after the ointment application and then hourly thereafter for 6 h. Skin irritation tests were performed with 5% or 10% Urea on the dorsal area (4 × 4 cm) of the rabbit with or without occlusive dressing. The animals were dosed twice daily for 10 days with 2 g of the test ointment. The skin was observed for adverse reactions at each dose.

The percutaneous absorption of 10% salicylic acid was significantly increased with the addition of 10% Urea. The control plasma value at 6 h was about 0.05 mg%, whereas the test material plasma value was about 0.15 mg% ($n = 4$). The absorption after 6 h for a 10% salicylic acid plus 10% Urea ointment was 9%, whereas the absorption for the control was about 1%. Urea derivatives with alkyl substitutions greatly enhanced the percutaneous absorption enhancing activity of Urea. Urea also increased the percutaneous absorption of the ionic form, sodium salicylate, of salicylic acid. Skin irritation tests with Urea (5% or 10%, one or both not specified) did not report any significant skin irritation on test animals within the 10-day treatment period (Han et al., 1991).

Babar et al. (1991) reported that a gel formulation that contained 6% Urea did not enhance the in vitro release of chlorpheniramine maleate through a cellulose membrane and hairless mouse skin model.

Lu et al. (1992) studied the effect of Urea on the permeability of a gonadotropin-releasing hormone. Skin was obtained from 6- to 8-week-old hairless nude female mice. The skin, excised from the abdomen, was mounted on diffusion cells and then preconditioned for 40 min. The surface of the donor side was fully covered with formulation when the experiment started. Human cadaver skin pieces from the thigh area was dermatomed to a thickness of 200 to 300 μm. The fully hydrated skin was mounted on diffusion cells in the flow-through system, and preconditioned for 40 min before application of the formulations. The skin was rapidly screened for barrier integrity by applying [^{3}H]water to the surface of the skin

A formulation containing 10% Urea alone in a vehicle containing ethyl alcohol/water did not enhance the permeability of leuprolide (gonadotropin releasing hormone) acetate (40 mg/ml) in human cadaver or mouse skin. In a formulation that contained 1% methanol, 1% camphor, 2% methyl salicylate, and 10% Urea in an ethanol/water vehicle nude mouse skin was 10-fold more permeable to leuprolide acetate than human skin (2.5 ± 1.40 ($\times 10^{-3}$) versus 2.2 ± 0.12 ($\times 10^{-4}$) cm/h. Permeability coefficients were determined 15 to 30 h after application (Lu et al. 1992).

Kim et al. (1993) performed two studies to determine the effects of Urea as a ketoprofen (nonsteroidal anti-inflammatory drug) penetration enhancer through excised skin from male Sprague-Dawley rats. Three different vehicles were used: 20% Urea plus 10.8 mg/ml ketoprofen in water, 10% Urea plus 210 mg/ml ketoprofen in propylene glycol (PG), and a 6%, 24%, or 36% Urea plus 8 mg/ml ketoprofen in a PG:ethanol:water (1:3:6) mixture. Each vehicle was used as a control. Ketoprofen concentrations for the water, PG, and mixture vehicle controls were 0.7, 170, and 8 mg/ml, respectively. The penetration of ketoprofen through excised rat skin for each vehicle was measured.

In all vehicles used, Urea decreased the lag time in the penetration of ketoprofen through excised rat skin; however, the effect of Urea on ketoprofen penetration through excised rat skin was dependent on the vehicle. The use of water as a vehicle plus Urea increased the diffusion parameter slightly, but decreased the permeation constant of ketoprofen significantly due to the large reduction in the partition parameter of the drug. However, when PG and PG:ethanol:water (1:3:6) mixture were used as the vehicles, a significant effect of Urea on ketoprofen permeation was not observed, although the results were statistically significant compared to controls. Urea in PG increased the diffusion parameter of ketoprofen about threefold compared to the control, which was compensated by a fivefold decrease in the partition parameter of the drug which resulted in a decrease in the permeation constant of the drug.

The addition of 36% Urea to the PG:ethanol:water mixture increased the diffusion parameter of ketoprofen by fourfold compared to the control PG:ethanol:water mixture. Although the partition parameter of the drug was decreased similarly by fourfold, an increase in ketoprofen penetration across the dermis layer was observed. In another experiment, a 20% Urea aqueous solution was applied to the excised rat skin mounted on the diffusion cell for 12 h. The control rat skin was pretreated with normal saline for 12 h. A ketoprofen aqueous solution excluding Urea was applied to the rat skin after removal of the Urea or saline solution. The diffusion cells for the permeation studies had an area of 3.14 cm^2. Fluid was withdrawn from the receptor phase at intervals up to 30 h.

Pretreatment with Urea increased the diffusion parameter significantly above control values, as did pretreatment with saline. The partition parameter was significantly lower for pretreatment with Urea compared to the control; however, pretreatment with saline increased the partition parameter over the control value. The permeability constant for pretreatment with Urea was significantly increased compared to control values. Pretreatment with saline caused an increase above both the control and pretreatment Urea values. The lag time was not significantly affected by either pretreatment, although both were lower than the control value and Urea was the lowest (Kim et al. 1993).

Kanikkannan et al. (1994) studied the in vitro absorption enhancement of indomethacin (another nonsteroidal anti-inflammatory drug) with 15% Urea (P1), 10% 1:1 Urea-octanol (P2), and 20% 3:1 Urea-PG (P3) incorporated in the patch formulation. Albino rat skin was excised from the abdomen. The dermal side of the skin faced the receiver fluid and the receptor compartment was filled with phosphate buffer pH 7.4. A circular transdermal patch (2.84 cm^2) was affixed over the hair free skin and the patch was covered with aluminum-foil as backing membrane. Each formulation was tested in triplicate.

The control (no enhancers, only indomethacin) had a steady state flux of 8 $\mu g/cm^2/h$ and had an enhancement factor of 1. Urea alone, P2, and P3 caused the flux of indomethacin to increase to 2.5, 3.25, and 3.75 times higher than control, respectively. The steady state flux of indomethacin was 20, 26, and 30 $\mu g/cm^2/h$ for Urea alone, P2, and P3, respectively. The flux of indomethacin containing Urea alone or in combination (P1, P2, or P3) took more than 10 h to reach steady state (Kanikkannan et al. 1994).

Chi et al. (1995) studied the penetration enhancing effect of 5% and 10% Urea, with and without 5% oleic acid, on 1% flurbiprofen (yet another nonsteroidal anti-inflammatory drug) in PG in rat skin harvested from male Sprague-Dawley rats and in vivo (four animals). Diffusion cells were fitted with a 5.1-cm^2 area of excised skin. The flurbiprofen plus Urea in PG solution (3 ml) was applied under occlusion to the epidermal surface and 0.2 ml of the receptor medium was withdrawn at 2, 4, 6, 8, 9, 10, 11, and 12 h. For the in vivo studies, the rats were anesthetized with ether and the hair on the dorsal area was removed with an electric clipper. Control and test vehicles that contained 1% flurbiprofen were uniformly applied (500 μl) to a dorsal area of 3×3 cm with gentle rubbing. Blood samples were collected from the jugular vein at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h post dose.

The permeation rate of 1% flurbiprofen in PG measured in rat skin harvested from male Sprague-Dawley rats was decreased with the addition of 5% and 10% Urea. Although 5% and 10% Urea in PG increased the diffusion coefficient of flurbiprofen about two times compared to the control PG vehicle, the 5 to 17-fold decrease in the partition coefficient of the drug compensated for the increased diffusion coefficient. This resulted in an overall decrease in the permeation rate of flurbiprofen; however, lag time was significantly shortened with the addition of Urea. The mixture of 1% flurbiprofen in 5% oleic acid plus 5% Urea in PG used to determine skin permeation in excised rat skin had a 1.7-fold higher permeation rate than oleic acid only and 9.7-times higher permeation rate then the control vehicle. Experimental data indicated that the increase in the permeation rate of flurbiprofen was due to the partition coefficient between the skin and vehicle and not the diffusion coefficient. Percutaneous absorption studies with oleic acid only and oleic acid plus Urea performed on the dorsal skin of rats reported significantly higher values for AUC, T_{max}, and C_{max} than the control (Chi et al. 1995).

Takahashi et al. (1995) studied the effect of Urea on absorption of an anti-inflammatory drug using an in vitro permeation study with abdominal skin from male Wistar rats. No details of the study were available. The authors stated that the flux of diclofenac from emulsion and cream formulations containing Urea was larger than that from the formulation without Urea.

Valenta and Wedenig (1997) used shaved skin from the abdomen of female rats to determine the penetration enhancement of progesterone by Urea. In vitro studies used Franz diffusion cells with an area of 0.785 cm^2. The stratum corneum of the skin faced the donor side of the diffusion cells. The composition of the formulation used was 3.0% progesterone, 92.0% unguentum polyethyleneglycol, and 5.0% Urea. The formulation (1.5 g) was placed on the skin surface of the donor compartment and sealed with parafilm. The receptor compartment was filled with PG-water, 40:60. The solution on the receptor side was maintained at $37^\circ C \pm 1^\circ C$ and stirred. Samples were collected from the receptor side at designated time intervals and replaced with fresh receptor solution. The permeation of progesterone from this formulation at 1, 7, and 24-h was 18.75 ± 9.9, 33.4 ± 8.44, and 69.5 ± 5.9 $\mu g/cm^2/g$, respectively. The addition of 5% Urea improved the permeation 2.5-fold after 24 h compared to the control (3.0% progesterone and 97.0% unguentum polyethyleneglycol).

Bentley et al. (1997) studied the influence of Urea on absorption of hydrocortisone. A gel was prepared with poloxamer 407 (25.0% *w/v*) in distilled water with 2%, 4%, 8%, or 12% *w/v* of Urea. Full-thickness skin was excised from the abdominal surface of HRS/J hairless mice and the stratum corneum was prepared. The available diffusion area of the diffusion cell was 2.54 cm^2 upon which 1.5 g of the formulations at an infinite dose was placed on the membrane. Hydrocortisone acetate (HCA) in PG was added at a concentration of 1.0% *w/v*. Samples from the receptor phase were withdrawn over 24 h.

The addition of Urea caused an increase in the lag time and a decrease in the flux of HCA with increasing concentrations of the penetration enhancer. The transfer of HCA from the formulation that contained 12.0% Urea did not occur until 12 h. As the concentration of Urea increased, an increase in the amount of HCA retained in the skin occurred accompanied by a decrease in the percutaneous absorption. Differential scanning calorimetry (DSC) showed no significant changes in the transition and enthalpy of the stratum corneum upon treatment with Urea. Fourier

transform infrared (FTIR) spectra from 2800 to 3000 cm^{-1} at room temperature and 30% to 60% hydration indicated that treatment with aqueous solutions of Urea (12.0%) induced small shifts of both asymmetric and symmetric C—H molecular bond stretching in the stratum corneum (Bentley et al. 1997).

Godwin et al. (1998) studied the effect of Urea on hydrocortisone absorption. Abdominal and dorsal skin of male hairless SKH1 mice were excised and used in an in vitro study to determine the penetration enhancement of Urea on hydrocortisone in PG over a 3.14-cm^2 area ($n = 5$). Control experiments were also performed with and without PG. Prior to application of 80 μl of hydrocortisone, Urea in PG was left on the skin for 1 h. Aliquots of the receptor phase were withdrawn over 24 h. The permeability coefficient for hydrocortisone plus Urea was 0.86 ± 0.16 cm/h (control $= 0.71 \pm 0.3$ cm/h; PG control $= 0.79 \pm 0.22$ cm/h). The receptor concentration after 24-h exposure to hydrocortisone plus Urea was 18.2 ± 3.0 μM (control $= 11.9 \pm 3.4$ μM; PG control $= 11.7 \pm 3.9$ μM). The skin content of hydrocortisone was 33.9 ± 7.5 μg/g (control $= 44.6 \pm 18.4$ μg/g; PG control 32.8 ± 20.2 μg/g).

Přiborsky et al. (1998) exposed male Wistar rats with bilateral nephrectomies to 2% concentrations of indomethacin, ibuprofen, and diclofenac (2-[(2,6-dichlorophenyl)amino] benzoacetic acid, monosodium or monopotassium salt, which is used for acute pain relief). Rats were anesthetized, placed on their back and had glass cells (16 mm inner diameter, 10 mm height) that contained the drug formulation (1.5 g) attached to the shaved skin with cyanoacrylate adhesive. Blood samples were taken from the jugular vein at 2.5, 5, 7.5, and 10 h after administration. Sham-operated animals served as controls. Further experiments were performed with indomethacin in a 50:50 ethanol-water solution with and without 10% Urea to determine solubility. The transdermal absorption was significantly reduced in all three cases under the condition of experimentally induced uremia compared to the controls. Urea (50 mM) was added to indomethacin in a gel ointment. The time-concentration profile of indomethacin was identical between control and treatment groups. Urea as a 10% formulation was also added to a gel ointment that contained indomethacin. A significant decrease in indomethacin serum concentration was observed. Solubility tests determined that indomethacin had a significant increase in solubility in the presence of 10% Urea (ratio of 1:2.5).

Skin Wound Healing

Troshev et al. (1990) measured the effect of Urea on skin wound healing in anesthetized Wistar rats that were shaved and given third degree burns over a 15-cm^2 area of the back. A propolis-Urea ointment was applied to the wound and the surrounding area immediately after the burn, then a trypsin compress with 3500 E trypsin and a compress containing the albuminous hydrolysate hydropot were applied to the damaged skin. This procedure was repeated for several days. It was not clear whether the propolis-Urea ointment and the compresses were applied to the same animals at the same time or to different animals at different times. Samples for microscopic evaluation were taken after 2 h, 3, 5, 7, and 14 days. After 2 h dystrophic changes in epidermal cells, sebaceous and sweat glands, and hair follicles were observed with demarcated inflammation under the muscle layer. On the 3rd day dystrophic changes in the epithelial cells and muscular fibers were increased. Dystrophic changes continued to the 5th day, circulatory changes were decreased and muscle fibers were invaded by granulation tissue.

The authors stated that regeneration was not evident by day 5, but was observed by day 7 as was well-developed granulation tissue. The epithelial layer was regenerated and differentiated by day 14. The dermal layer had mild hyperemia with some corresponding inflammatory reactions. The striated muscle contained granulation tissue and the inflammatory reaction was most intense at the dermal and epidermal demarcation lines (Troshev et al. 1990).

Grogl et al. (1999) used a 10% Urea formulation to facilitate the penetration of 15% paromomycin (an aminoglycoside antibiotic) to treat murine cutaneous leishmaniasis in hamsters and mice. Lesions treated with this formulation healed by day 20 as with other treatments; however, 30% relapsed by day 50.

Effects of Urea on Collagen

Rose and Mandal (1996) studied the interaction of catfish collagen with Urea at various temperatures. The SDS–polyacrylamide gel electrophoresis (PAGE) pattern compared to a standard of rat tail tendon collagen indicated that catfish collagen is type I in nature. The ratio of proline to hydroxyproline is 1:2 which suggested that catfish collagen is vertebrate. The molecular weight of catfish collagen is 320,000 daltons and the shrinkage temperature is 54.5°C.

Collagen was exposed to 2 M Urea at temperatures of 20, 25, 30, 35, 40, 45, and 50°C and had hydrated specific volumes (V_h) that ranged from 0.40 to 0.59 ml/g, whereas the buffer had a V_h that ranged from 1.06 to 2.30 ml/g. The intrinsic viscosity factor (n) from lowest to highest temperature ranged from 5.3 to 2.65 dl/g for Urea, whereas the buffer ranged from 12.7 to 0.80 dl/g. The asymmetry factor (v) for Urea ranged from 662.4 to 359.9, with nothing determined at the highest temperature, and the buffer ranged from 1049.6 to 74.5, with nothing determined at the two highest temperatures.

The investigators concluded from this data that the V_h of catfish collagen and buffer increased with increasing temperature up to 25°C and 35°C, respectively, but beyond those temperatures an opposite trend was observed regardless of the environment. Urea also contributed to hindrances in the stabilization of this type of collagen. The formation of an aggregated core of collagen around Urea (1 M) is not possible even above the denaturation temperature of collagen. However, in the presence of a high concentration of Urea (6 M) and above the denaturation temperature of collagen, it is possible for collagen to be incorporated into a micellar core of Urea, which might be due

to the peculiar condensing properties of Urea. The denaturation temperature for both collagen in buffer and 1 M Urea was 30°C. At 1 M Urea, a sudden decrease in catfish collagen viscosity occurred and remained somewhat constant. The viscosity fell rapidly between 3 and 6 M Urea and remained constant from 6 M onward, which indicated a complete denaturation at about 6 M. At concentrations of 1, 2, 3, 4, 6, and 8 M Urea there was a gradual increase in the percent reduction in viscosities from 81.0% to 93.1% (Rose and Mandal 1996).

Effects of Urea on Stratum Corneum

Elfbaum and Wolfram (1970) exposed isolated guinea pig stratum corneum to 6 M Urea for 24 h. The changes in mechanical properties were evaluated by the work index, which was determined by comparison of 5% stretching in solvent vs. initial 5% stretching in water from the skin of two animals. Urea considerably weakened the stratum corneum; however, the action was partly reversible. Reversibility was assessed by rinsing the tested strips with distilled water, relaxing them overnight in $CHCl_3$ saturated water, and restretching them a third time in water. The initial work index in water was 0.90 ± 0.16 and the work index in the solvent was 0.73 ± 0.16.

Effects of Urea on Other Molecules

Reske and Bauer (1973) studied the effects of 6 M Urea and 0.5% caffeine on the photooxidation of tryptophan and 3,4-benzopyrene in aqueous solution. In the presence of Urea, an increase in the absorption bands of 3,4-benzopyrene photoproduct was observed and the absorption of the tryptophan photoproduct remained unchanged at 366 nm. No significant difference was observed at 335 nm when Urea was added to the aqueous solution of tryptophan, 3,4-benzopyrene and caffeine. The photocooxidation of 3,4-benzopyrene and tryptophan in solutions of dodecylsulfate is replaced by a benzopyrene-photosensitized oxidation of tryptophan with the addition of 6 M Urea. The quantum yield of 3,4-benzopyrene photoreaction in soap solutions of 0.45% sodium dodecylsulfate plus 6 M Urea was 0.49×10^{-3} and 0.34×10^{-3} with the addition of tryptophan. The control value for 3,4-benzopyrene alone was 0.22×10^{-3} and with the addition of tryptophan the value was 0.76×10^{-3}. Urea did not change the benzopyrene spectra when added to a solution which contained caffeine.

In an in vitro study, Kappen and Goldberg (1979) examined the effects of Urea on neocarzinostatin (NCS), an antitumor antibiotic which also inhibits DNA synthesis, causes DNA strand breakage, induces DNA repair synthesis in whole cells and in isolated nuclei, and introduces single-strand breaks exclusively at thymidylate and adenylate residues in DNA. Reaction mixtures contained 0.8 μg of λDNA (2.4×10^4 cpm), 100 μl/ml NCS, and 0.1 to 10 M Urea and were incubated for 30 min at 37°C. Urea enhanced the DNA cutting activity of NCS over the 0.1 to 10 M Urea concentrations. Additionally, preincubation of NCS with 4.8 M Urea in Tris (tris(hydroxymethyl)aminomethane) buffer at pH 8.0 before the addition of DNA produced an "appreciable" decrease in the activity of the drug.

Valentovic and Bachmann (1980) studied the effects of Urea on hexobarbital and antipyrine disposition in female albino Sprague-Dawley rats. Animals were dosed according to the following schedule: 26 mg/kg IP twice daily for 3 days, 26 mg/kg IP twice daily for 10 days, or 500 mg/kg IP twice daily for 3 days. Control animals received equivalent volumes of 0.9% sodium chloride accordingly. Hexobarbital (65 mg/kg) was administered IP 1 h after the last pretreatment injection and blood was drawn by cardiac puncture 15 min after this dose. Antipyrine (100 mg/kg) was administered IP 1 h after the last pretreatment injection. One hour after the last pretreatment injection, animals pretreated with saline or high-dose Urea were injected IP with [^{14}C]antipyrine (15 mCi/mmol), which was also diluted with unlabelled antipyrine for a total dose of 50 mg/kg. The total reactivity administered was 20 μCi/kg. Blood samples were taken by cardiac puncture at 0, 2, 4, 6, 8, and 10 h after antipyrine injection.

In vitro metabolism studies used livers from Urea-pretreated rats, saline-pretreated rats, or naive (nonpretreated) rats. Rats pretreated with Urea were killed 1 h following the last pretreatment injection. Livers were processed and 1 mg of hexobarbital was added. Urea in concentrations of 2.5, 5.0, and 10 mmol/g equivalent liver was added to incubation flasks prepared from the livers of naive rats. Flasks were incubated for 10 min prior to and 5 min after hexobarbital addition.

Animals pretreated with Urea at 26 mg/kg twice daily for 10 days or 500 mg/kg twice daily for 3 days had shortened duration times for loss of the righting reflex and lengthened sleep times compared to the control. Livers from pretreated rats metabolized hexobarbital more slowly than those from saline controls, while microsomal protein and cytochrome P-450 were not significantly reduced following Urea pretreatment. The direct addition of Urea to liver fractions excised from naive animals produced a concentration-dependent change in in vitro hexobarbital metabolism, cytochrome P-450 content, and microsomal protein. Although hexobarbital metabolism was progressively inhibited with increased Urea concentrations, cytochrome P-450 was not significantly decreased until 5 mmol Urea/g equivalent liver added. Cytochrome P-450 could not be detected with 10 mmol Urea/g equivalent liver added. Microsomal protein was reduced by 89% at this concentration.

Temperature depression was comparable between control animals and those pretreated with Urea. The Urea pretreatment by either schedule shortened the duration of antipyrine-induced temperature depression; the duration of temperature depression after the low- and high-dose Urea pretreatment was 50% and 41% shorter than saline-pretreated controls, respectively. Single doses of Urea (500 mg/kg) did not produce a significant hypothermic effect. A semilogarithmic plot of the disappearance of [^{14}C] antipyrine from whole blood of animals pretreated with saline or high-dose Urea (mean value of three rats) determined the half-life of antipyrine as 3.1 and 1.7 h for saline-pretreated

and Urea-pretreated animals, respectively (Valentovic and Bachmann 1980).

El-Sourady et al. (1986) used male hooded Wistar rats to study the diffusion rate of aspirin, salicylamide and phenacetin through the rat's ileum. Aspirin was prepared in a solid dispersion with Urea in Ringer's solution at pH 4 so that a 1 mg/ml concentration of aspirin was present in each carrier. The same procedures were adopted to prepare 1 mg/ml solutions of salicylamide in Ringer's solution adjusted to pH 5 and 0.5 mg/ml phenacetin in Ringer's solution at pH 6. The rats were fasted for 20 to 24 h with water available ad libitum. The rats were killed by a blow to the head. The distal portion of the small intestine was discarded and small equal segments from the rat intestine were used. The intestine sac was filled with 1 ml/5 cm segment of the drug solutions and the ends were tied off. The segment was immersed in a measuring cylinder with 25 ml Ringer's solution. The measuring cylinder and its contents were immersed in a water bath at 37°C and its contents were oxygenated. Samples (1 ml) from the organ bath were withdrawn at 10-min time intervals. Each experiment was repeated four times.

Urea highly increased the diffusion rate of aspirin, but decreased that of salicylamide and phenacetin. The area under the diffusion rate curves (AUC) at 10 to 60 min for aspirin, salicylamide, and phenacetin were, respectively, 3000, 1112, and 659. The respective control AUC values were 2710, 1449, and 1246 for aspirin, salicylamide, and phenacetin (El-Sourady et al., 1986).

The release of testosterone from a water washable base with 3%, 5%, or 10% Urea was evaluated in vitro by Parikh et al. (1986). Jars with a surface area of 6.83 cm^2 were each filled with a testosterone plus Urea formulation. The jars were covered with a semipermeable cellophane membrane which had been soaked in phosphate buffer for 6 h. The jars were inverted and immersed in a 250-ml beaker that contained phosphate buffer. At 5, 15, 30, 45, 60, 90, and 120 min aliquots were drawn off. The 10% Urea formulation added to the water washable base increased the release of testosterone from the base by 50%. The other additive concentrations had no effect on the release of testosterone from the base.

Nascimento and Sakate (1996) reported that the depressive effect of carbofuran on contractile force and frequency of spontaneous beating in isolated guinea pig atria was reversed by the addition of 6.0 mg/ml Urea.

Cytotoxicity

Schneider et al. (1969) added Urea in concentrations of 100 to 500 mg% to thrombocytes which resulted in a remarkable decrease in oxygen uptake and ATP content. Neither the glucose consumption nor the formation of lactate was altered in the presence of Urea. A small decrease in pyruvate formation occurred. The investigators explain that the diminished content of ATP in the platelet cannot be explained by inhibition of glycolysis; it depends on oxidative metabolism, a sensitive parameter for thrombocyte formation.

Szász and Gárdos (1970) determined that small concentrations of Urea (0.4 to 0.8 M) induced significant changes in the energy metabolism of erythrocytes. (Details of the protocol used were not included.) A prompt and significant decrease in the ATP level without the release of inorganic phosphate at the same time occurred.

Wardle (1970) studied the effect of various metabolites commonly elevated in uremia, including Urea, on red blood cell metabolism. Red blood cells were incubated in the presence of 250 mg/100 ml Urea at pH 6.8, 7.4, and 7.8. There was no change in reduced glutathione content of red blood cells with Urea treatment at pH 6.8 for 2 h or overnight, at pH 7.4 for 2 h or overnight, but there was a one standard deviation (from the controls value of 60 ± 10.5 mg/100 ml) increase at pH 7.8 for 2 h. Uptake of [^{32}P] orthophosphate into red cells incubated with Urea (concentration not reported) for 2 and 4 h was greater than control uptake at 4 h, but not at 2 h. Methemoglobin formation, calculated as a percentage of hemoglobin produced, was comparable to controls after 2 h of incubation with Urea. Pyruvate kinase and glutathione reductase activities were reduced in red blood cells after 2 h of incubation with Urea. ^{42}K uptake in red blood cells treated with Urea for 2 hours was larger than controls, but between 2 and 4 h of treatment with Urea, the potassium uptake was the same as the control values. The authors concluded that Urea produces minor changes in red blood cell metabolism, but has no great toxicity.

Glinos et al. (1983) exposed HeLa cells in culture, with a doubling time of about 26 h and supplemented with 20% horse serum and 100 μg/ml gentamicin, to 0.5%, 1.0%, 1.4%, 1.5%, or 5.0% Urea from 24 h to 14 days. The results presented in Table 7, from cultures incubated for 24 h, demonstrate a marked increase in the mitotic index in Urea treated cultures. The investigators concluded that the mitotic blocking action of Urea was unrelated to any nonspecific osmotic effects, since the increase in mitotic index was associated with a marked inhibition of growth.

Accumulation of mitotic figures began at 4 to 6 h after exposure to 1.4% Urea, peaked at 15 to 18 h and declined progressively thereafter. The results also indicate that after 15 to 18 h, the rate of cell entry into mitosis is lower than the rate at which the cells escape from mitotic block. The block decreased slowly over time, which indicated that the return of the mitotic index to normal levels after 21 h was due to a second, later effect of Urea: a decrease in the entry rate of cells into mitosis by a delay in their passage through the cell cycle phases preceding mitosis. Continuous exposure to Urea up to 12 days resulted in mitotic indices that did not exceed those of the control culture.

In another experiment by these authors, HeLa cells were exposed to an initial 1% Urea concentration. The medium was not renewed for the first 3 days, but the concentration of Urea was subsequently increased to 1.2% and the media was renewed every 48 h and the cell density remained $<10^6$ cells/ml throughout the experiment. The 5-day control cultures had a mean viability value of 99.4%. In experimental cultures, viability was 99.6% for the first day and averaged 98.2% for the interval 2 to 12 days.

TABLE 7
Effects of media made hyperosmolar with Urea on HeLa cells (Glinos et al. 1983)

Urea concentration (%)	Medium osmolarity (mos M)	Initial cell density, cells/ml $\times 10^{-3}$	Final cell density, cells/ml $\times 10^{-3}$	Growth index	Viability (%)	Mitotic index (%)	Microscopic morphology
5	1125	222	176	0.79	91.2	0	Cell shrinking; extensive clumping of viable and dead cells; cytolysis and large amount of cellular debris
1.5	540	211	211	1	93.7	9	Cell shrinking; cytolysis and large amount of cellular debris; abnormal cellular shapes and membranes and hyalinized cytoplasm; increased no. of abnormal mitoses as compared to control culture
1.4	523	250	285	1.14	99.4	11.42	Cell shrinking; increased no. of abnormal mitoses as compared to control culture
1	457	185	240	1.29	99.7	4.9	Abnormal cellular shapes and membranes and hyalinized cytoplasm; increased no. of abnormal mitoses as compared to control culture
0.5	373	177	293	1.65	99.7	2.92	Cellular morphology identical with that of control culture
0	0	181	329	1.82	99.9	2.89	Cellular morphology identical with that of control culture

The average frequency of distribution of the mitotic phases during the control culture's 5 days of growth were: prophase plus metaphase, 79.3%; anaphase, 12.7%; and telophase, 8.0%. The first 12 days of the Urea-treated culture had an average frequency of distribution for prophase plus metaphase, 84.6%, anaphase, 6.5%, and telophase, 8.9%. Continuous exposure to Urea caused an attenuation of the metaphase block. The growth of the Urea-exposed culture was markedly inhibited, but was without a decrease in cell population.

These authors also performed experiments to obtain information about cell cycle phases in which alternate delay and acceleration of the cells generated peaks of arrested mitosis. The fraction of ^{3}H-labeled cells and [^{3}H]dThd incorporation into total DNA under conditions of intermittent or continuous exposure to Urea were determined. Urea was added at a concentration of 1.4% at time 0 and 57 h with media renewal, whereas at time 33 and 81 h Urea was not added and the media was only renewed. Two peaks of arrested mitosis occurred at 17 and 73 h, although the peak at 17 h also occurred with a decrease in the fraction of labeled cells which also occurred in the control cultures and was attributed to nutrient limitation. The relative frequencies of the mitotic phases at the two peaks of the mitotic index curve were at 17 h: prophase plus metaphase, 98.6%; anaphase, 0.7%; telophase, 0.7%, and at 73 h were prophase plus metaphase, 98.0%; anaphase, 1.1%; and telophase, 0.9%. At the lowest point on the curve the relative frequency of prophase plus metaphase was 93.7%; anaphase, 4.2%; and telophase, 2.1%. The growth index decreased progressively after the addition of Urea, but just after the mitotic peak, the index rose to a maximal value and was followed after 15 h by a rise in the labeling index and [^{3}H]dThd incorporation into total DNA, regardless of whether Urea was present or not in the media after 33 h.

Finally, these authors compared the growth of five tube HeLa cell cultures in media containing 1.0% Urea to the growth of five control cultures after a 3-day incubation without renewal of the media. The initial total protein value per tube was 64 μg and cell attachment was verified microscopically. At the end of the 3-day growth period, total protein values per tube for the Urea-treated cultures and the controls had a mean of 92 μg and 380 μg, respectively. The 3-day factorial increase of the

protein content per tube in the Urea-treated cultures and the controls was 1.44 and 5.9, respectively. In a comparable 3-day experiment performed in suspension cultures without medium renewal, similar results were obtained. Thus, whether in attached or suspension cultures, treatment with Urea at a concentration of 1.0% resulted in a 70% to 75% reduction in HeLa cell growth (Glinos et al., 1983).

Phelps et al. (1983) examined cell-cell interactions between a mouse fibroblast cell line (10T½) and a chemically transformed daughter line (MCA) exposed to Urea and Urea isolated cell-surface protein (CSP). The effects of 0, 50, 100, 200, and 300 mM Urea exposure for five days on growth and overlap index were determined for both cell lines.

Both cell lines demonstrated a progressive inhibition of DNA synthesis with increasing concentration. Protein synthesis was strongly inhibited in the MCA cell line at all Urea concentrations; however, in the 10T1/2 cell line, protein synthesis was inhibited only at the highest Urea concentration. An overlap analysis was performed in which 5-day cultures were fixed for 30 min in 10% formalin, placed in 70% ethanol overnight, stained and rinsed, and then the number of nuclear overlaps were scored. The 10T1/2 cells at 300 mM Urea and the MCA cells at 200 and 300 mM Urea did not grow sufficiently for overlap analysis. The 10T1/2 cell-overlap slopes increased in the presence of Urea while the MCA slopes decreased (Phelps et al., 1983).

Dierickx (1989) incubated Hep G2 cells for 24 h with five different concentrations of Urea. The PI_{50} (the concentration of test compound required to induce a 50% reduction in cell protein content) of Urea was determined to be 279 mM.

Sato et al. (1990) induced an increase in both cytosolic Ca^{2+} and prolactin secretions in GH_4C_1 cells Isotonic with Urea in a medium with normal 1.2 mM Ca^{2+} concentration. Each peak was proportional to the concentration of Urea between 5 and 120 mM. A significant linear relationship between these induced peaks also occurred. The removal of Ca^{2+} from the medium completely abolished the increase in Ca^{2+} and prolactin secretion. Hypertonic Urea was ineffective in inducing either an increase in Ca^{2+} or prolactin secretion.

Maddock and Westenfelder (1996) studied the induction of heat shock proteins by Urea. SK-N-SH human neuroblastoma cells were exposed to 20, 40, 60, 100, 150, and 200 mg/dl Urea for 5 h. The lowest concentration of Urea did not induce heat shock proteins (indicative of cytotoxic damage). Concentrations of 40 to 200 mg/dl, however, resulted in the production of one heat shock protein, Hsp72. The intensity of expression of Hsp72 increased about 1.7-fold up to 150 mg/dl Urea and then leveled off. To determine the length of duration and pattern of heat shock response to Urea, 200 mg/dl Urea was added to quiescent test cells maintained in an incubator for 30 min, 1, 5, 10, 24, 33, and 48 h. Control cells were harvested at 48 h and Hsp72 was not present. Hsp72 was present 30 min after the addition of Urea; it increased and peaked in intensity up to 10 h and gradually declined and was gone by 48 h after the addition of Urea.

Other Urea Biological Activities

Harper and Bell (1963) conducted a study in which the brain cortex of five mongrel dogs was exposed and cannulae were placed in the saggital sinus, the superior thyroid branch of the carotid artery and the femoral artery. The latter cannula was connected to a mercury manometer. After three initial control measurements at 20-min intervals of blood flow and oxygen uptake, 1.5 g/kg lyophilized Urea in 10% invert sugar (Urevert) was administered over 10 min. Estimations of blood flow and oxygen uptake were made at 5, 30, and 60 min. A dramatic shrinkage of the brain occurred within 5 to 10 min of administration of Urea, which persisted for about an hour and then began to recover to its normal volume. The brain shrinkage was accompanied by a transient rise in blood pressure. Four dogs had a rise in arterial pCO_2; however, 30 min after administration of Urea the pCO_2 returned to control levels. No significant changes occurred with respect to blood flow or oxygen uptake after the administration of Urea.

Gregory and Robinson (1965) incubated slices of liver from adult male hooded rats in 0 to 10 M Urea. Experiments with nonrespiring slices incubated in 0 to 3 M Urea for 4 h in 1 ml of ordinary saline medium (Na^+ 145 mequiv/L; K^+ 5 mequiv/L; Ca^{2+} 5 mequiv/L; Mg^{2+} 2 mequiv/L; Cl^- 140 mequiv/L; SO_4^{2-} 2 mequiv/L; and buffered with 13.5 mequiv/L phosphate at pH 7.4). A total of 11 samples were used. Experiments with respiring slices used a medium containing 100 mg/ml glucose and graded concentrations of Urea up to 10 M. Oxygen consumption was measured 1 h after the initial 15-min period of equilibrium. Water content was determined as the loss of weight after drying for 16 h at 80°C.

Oxygen consumption of slices incubated in ordinary medium ranged between 1.5 and 1.9 μl/mg initial moist weight. However, the initial moist weight was inflated by about 35% during the initial stirring in the medium at room temperature. The normal value for tissues incubated in phosphate saline is 2 μl/mg. Oxygen consumption was markedly depressed by Urea concentrations up to 2 M. Little change occurred at increased Urea concentrations. Persistent oxygen uptake was more reduced by boiling the liver slices than with Urea exposure.

Control experiments were contaminated with bacteria and, therefore, showed no oxygen uptake. The steep increase in tissue hydration above 1 M did not cause a marked change in respiration. Liver slices exposed to 2 to 5 M Urea were softened and had lost some of their color. Those incubated at higher concentrations were leathery, easy to blot and had a deep green-brown color. A steep fall in tissue potassium and a concomitant rise in sodium content occurred as the concentration of Urea increased from 0 to 2 M. However, no further changes occurred at higher concentrations of Urea.

The intracellular osmolality (indicated by the Na^+, K^+, and Cl^- concentrations) remained fairly constant at about 300 mEquiv/kg water as the Urea concentration increased to 1 M. As the Urea concentration increased to 5 M it remained steady.

At Urea concentrations of about 1 M, an abrupt change in tissue water content was accompanied by a corresponding change in the quantity of sodium in the tissue and the loss of potassium from the tissue was shown to be absolute. The reduction in water content with concentrations above 2 M may have accompanied the reduction in sodium content. The fixed anionic charges in the tissue (the concentration of Na^+, K^+ and Cl^-) remained constant at about 240 mequiv/kg as the Urea concentration increased to 2 M, but decreased to 140 mequiv/kg in 5 M Urea. The pH of the medium did not correlate with this change. Concentrations of Urea below 2 M did not affect the water content of the tissue. Liver slices incubated at 0°C had increased water content at Urea concentrations greater than 2 M (Gregory and Robinson, 1965).

Rapoport et al. (1972) applied topical Urea (2.2 to 9.6 molality) to the cortical region of 127 rabbits for 10 min. Another 33 rabbits were injected with Urea in the carotid artery. Evans blue was injected into the femoral vein before and after exposure to Urea. Both methods of exposure to Urea produced reversible barrier damage to the brain. The intracarotid perfusion revealed that injection of the dye before infusion of Urea caused unilateral hemispherical damage in the region supplied by the internal carotid artery, but no dye extravasation was evident when the dye was injected 30 min after exposure to Urea.

Brightman et al. (1973) reported that a 3-M hyperosmotic Urea solution dosed IV or applied topically to the pia-arachnoid surface of rabbits for 90 s to 6 min opened up the endothelial tight junction barrier to the passage of peroxidase from blood to cerebral extracellular fluid.

Pollay (1975a) reported that the injection of adult mongrel dogs (21) with 10 ml of 3 M Urea into the vertebral artery over a 20- to 30-s period enhanced the permeability of the blood-brain barrier to glucose and fructose and enhanced cerebral blood flow.

Pollay (1975b) reported that the blood-brain barrier in one cerebral hemisphere of six rabbits was damaged by ipsilateral infusion of hypertonic 3 M Urea into the carotid artery. In the Urea infused cerebral hemisphere, Urea enhanced the clearance of glycine and phenylalanine by simple and facilitated diffusion, respectively.

MacKenzie et al. (1976) gave six young baboons intracarotid injections of 7 to 10 ml of a 2-osM Urea solution over 15 s. No significant differences were observed for oxygen consumption by arterial blood, cerebral oxygen consumption, cerebral glucose uptake, cerebral blood flow, and mean arterial blood pressure. The brains of animals injected with Evans blue prior to administration of hypertonic Urea were heavily stained at necropsy. Six animals injected with Urea, as before, plus 50 ng/kg/min norepinephrine had significantly increased values for cerebral blood flow and oxygen and glucose consumption compared to baseline values.

Spatz et al. (1976) perfused New Zealand rabbits through the intracarotid artery with hypertonic 2 and 3 M Urea and 0.3 M Urea adjusted to pH 7.4. The animals had increased brain uptake indexes of glucose analogues, while isotonic Urea was without effect.

Pickard et al. (1977) injected a hypertonic solution of 2 M Urea into the intracarotid artery of the baboon to disrupt the blood-brain barrier. Penicillin G and Evans blue albumin were administered to three and four animals, respectively. Penicillin G, normally without effect on the electroencephalogram (EEG), affected the paroxysmal spike discharges on the EEG. Evans blue stained the area of the brain infused with the dye.

Luduena et al. (1987) used Urea in studies of the interaction of a microtubule assembly inhibiter with tubulin. Tubulin, prepared from bovine cerebra (0.66 mg/ml), was incubated for 1 h at 37°C with 1.36 mM iodo-[^{14}C]acetamide in the presence and absence of 8 M Urea and in the presence and absence of a 50 μM concentration of 2-(4-methyl-1-piperazinylmethyl) acrylophenone dihydrochloride (MPMAP), colchicine, or vincristine. MPMAP is a potent inhibitor of microtubule assembly in vitro and a weak inhibitor of colchicine binding to tubulin. Studies with Urea were carried out to characterize and categorize more fully the interaction of MPMAP with tubulin.

Colchicine and vincristine had increased radioactivity in the presence of 8 M Urea compared to the controls without Urea. MPMAP inhibited the alkylation of tubulin by 97% in the presence of 8 M Urea; the stoichiometry suggested that each sulfhydryl group was now accessible to MPMAP. At a decreased concentration of MPMAP (15 μM), the inhibition of alkylation of the tubulin in the presence of 8 M Urea was not as great as in the absence of 8 M Urea. MPMAP (15 μM) was also incubated with aldolase (0.66 mg/ml) in the presence and absence of 8 M Urea. MPMAP inhibited the alkylation of aldolase by iodo-[^{14}C]acetamide both in the presence and absence of 8 M Urea. Inhibition of alkylation in the presence of 8 M Urea was equal for tubulin and aldolase; however, in the absence of Urea, the alkylation of tubulin was inhibited more strongly than that of aldolase (Luduena et al. 1987).

Gordon (1989) studied thermoregulatory and behavioral responses in eight male BALB/c mice per group injected once IP with 1000 or 4500 mg/kg Urea and a control group (although some graphical data implied that mice were dosed up to 6000 mg/kg). The injected mouse was placed in a chamber thermostabilized to 20°C ± 1.0°C. One hour after injection the mouse was removed and its colonic temperature was measured. Behavioral experiments involved placing mice in a temperature gradient for 90 min after injection with Urea. Position was converted to temperature every two minutes by using a standard calibration curve of position versus temperature. Urea had no effect on body temperature up to a dose of 3280 mg/kg, whereas the colonic temperature decreased beyond this dose. Additionally, 1000 and 4500 mg/kg Urea had little effect on behavioral thermoregulation. All mice survived the highest doses of Urea for the duration of the experiment.

Heinz et al. (1990) used a UV absorption maximum shift and enzyme activity to study the effect of Urea on the enzyme liver alcohol dehydrogenase (LADH). The emission maxima for the native form of LADH is 334 nm, due to tryptophan residue fluorescence. After denaturation with 9 M Urea for 24 h at 4°C,

the maximum shifts to 346 nm (a 12-nm red shift), indicative of a change in the tryptophan conformation, i.e., unfolding. In two other experiments, LADH was denatured in 4.85 M Urea at 30°C. Emission spectra were collected and the activity was assayed at various times. The activity of LADH and fluorescence decayed exponentially as a function of time of exposure to Urea. The lowest activity was obtained after a 42-min exposure to Urea.

Gordon et al. (1995) studied the effect of Urea on the intracerebral arterioles. Solutions of Urea (0 to 100 mM) in a balanced salt solution (pH 7.3, 37°C) were applied to the arterioles and vessel diameter was recorded after 5 to 7 min of equilibration. Arteriole diameter increased after exposure of the vessel to the highest dose of test agent. A significant difference was observed between the difference in end tone versus basal tone. Urea also caused a dose-dependent vasodilation. Although exposure to Urea caused a loss of basal tone, the vessel's ability to contract in response to a pH increase remained intact.

Takeuchi et al. (1995) performed studies that measured simultaneously the gastric transmucosal potential difference (PD) and luminal pH in anesthetized male Sprague-Dawley rats. The stomach was initially perfused until the PD and pH were stabilized, the perfusion was stopped and the following agents were applied topically to the mucosa for 10 min: Urea (0.1% to 0.5%), urease (50 to 200 units), and Urea plus urease. The mucosa was rinsed gently and the perfusion resumed after application. Ulcerogenic activity was evaluated orally through the administration of 1% to 6% Urea plus 100 units urease. The animals were killed one hour later and the macroscopically visible lesions were measured. The protective activity was measured in animals by oral administration of 60% ethanol in 150 mM HCl.

The following reagents were given orally 30 min before administration of HCl/ethanol: 1% Urea, 100 units urease, and 0.2% to 1% Urea plus 100 units urease. In some experiments, the animals were given 5 mg/kg indomethacin (nonsteroidal anti-inflammatory drug) SC 30 min before administration of 1% Urea plus 100 units urease.

The mucosal exposure to 0.5% Urea or 100 units urease for 10 min did not cause a change in PD and pH; however, the combined application of >0.1% Urea plus 100 units urease produced a reduction of PD and an increase in pH. This response was increased in a concentration-dependent manner for Urea and urease, and reached a maximal level at 0.5% Urea plus 100 units urease. Intragastric administration of Urea and urease, alone and in combination, did not cause any macroscopic lesions in the gastric mucosa, even at 6% Urea administered with 100 units urease.

Oral administration of HCl/ethanol caused severe hemorrhagic lesions in the gastric mucosa. Animals pretreated with various concentrations of Urea (0.2% to 1%) with 100 units urease had a decrease in the severity of the lesions in a concentration-dependent manner. A significant effect was observed at 0.5% Urea plus 100 units urease. The development of gastric lesions in response to HCl/ethanol was not significantly affected by 1% Urea or 100 units urease separately. The gastroprotective action of combined treatment with 1% Urea plus 100 units urease was significantly decreased compared to controls by prior treatment with indomethacin (Takeuchi et al. 1995).

ANIMAL TOXICOLOGY

Acute Toxicity

Blake et al. (1976a) used adult pregnant and non-pregnant adult female rhesus (*Macaca mulatta*) monkeys to study the effects of hyperosmolar Urea. Intravenous infusion of 5% dextrose in 0.45% saline was maintained at a rate of 0.3 to 0.5 ml per minute. Urea was prepared for injection by adding 40 ml of 5% dextrose in water to 40 g of sterile Urea. [^{14}C]Urea was added to the nonradioactive Urea solution so that the injected dose contained about 25 μCi of radioactivity. Urea was injected over 3.5 min. A dose of Oxytocin (25 mU/min) equivalent to that used in human abortions was administered immediately after the Urea injection. Urine and blood were collected on days 1, 4, and 7 post treatment. Blood was also collected prior to Urea injection.

Two monkeys died 20 to 24 h after receiving injections of 1.8 g/kg IP. Urea and 12.5 g/kg intra-amniotic (IA) Urea, respectively. All surviving animals were healthy at the 3- to 6-month evaluation.

[^{14}C]Urea was rapidly absorbed after ip injection; the serum concentration peaked within 30 min and nearly reached the peak measured after IV injection. The average IV concentration of Urea was 375 mg/dl 4 min after the end of a 3.5-min injection. As venous concentrations of Urea decreased exponentially, the half-time was 5 to 6 h. A similar rate of elimination was observed for IP and IA injections. The apparent volume of distribution was 70% to 85% of body weight.

IV and IP injections of Urea caused maximum diuresis initially at about 20 min after the start of the injection and then it tapered off to the flow rate of the dextrose/saline infusion. Urea clearance by the kidney showed a parallel increase during the period of diuresis.

IA injections caused urine flow and Urea clearance to increase steadily and plateau at about 2.5 h. Urea clearance was significantly higher 3 to 4 h after IA injection (6.1 ± 0.3 ml/min) than after combined IV and IP injection (4.4 ± 0.6 ml/min). The only remarkable change in serum electrolytes was a decrease in serum potassium in monkeys that received systemic injections of Urea. [^{14}C]Urea in urine had a cumulative 24-h excretion of 60% to 85% of the IV dose, 70% to 110% of the IP dose, and 35% to 60% of the IA dose. Marked hemolysis and hematuria were observed 1 to 2 h after IV injection, but hematocrits were not different from those of the IP group. The total white cell count increased from 50% to 200% within 24 h in all monkeys. Transient leukocytosis in the IA group persisted for about 7 days after the IV and IP injections of Urea (Blake et al. 1976a).

Teramoto et al. (1981) dosed four female rats orally once with 2000 mg/kg Urea and six female mice were dosed orally once with 1000 or 2000 mg/kg Urea (3/group). The animals were examined for mortality or toxicity for 1 week. None of the animals died and toxicity was not observed at any dose.

Thurston et al. (1986) injected 65 mice (17 to 23 days old) with 1 M NaCl or 2 M Urea. An elevated plasma Na^+ concentration and osmolality, as in acute hypernatremia, was achieved by injecting the mice with 30 ml/kg Na^+ SC in the back and another 30 ml/kg IP. Weight-matched littermates received equivalent volumes of 0.9% NaCl. Mice were killed by decapitation at selected postinjection times and blood was collected.

Salt-loaded mice exhibited the following behaviors within minutes after injection: staggering, hopping, running in circles, head-shaking, walking on toes, and hypersensitivity to touch. Most of the mice were splayed 30 to 60 min after injection, the normal arch to the back was lost and no movement was observed. Clinical improvement was not observed until 2 h after injection. Urea-treated animals had the same abnormal neurologic signs soon after injection; however, there was rapid improvement by 1 h. None of the animals treated with Urea had splaying or paralysis. Urea-treated animals lost 10% of their body weight by 6 h compared to an 11% loss by the salt-loaded mice and a 5% loss by the 0.9% NaCl-injected controls.

At gross evaluation of the brain there was a hemorrhagic encephalopathy 1 h after treatment in Urea and salt-loaded animals. No microscopic evaluations were made. Urea-injected mice had significantly decreased plasma Na^+ concentrations compared to controls during the first 2 h after injection and significantly higher concentrations than controls after 2 h. Injection of salt or Urea did not change plasma K^+ concentration during the 6-h experimental interval. Plasma osmolalities were still higher than in controls 6 h after injection.

After 0.5 h the brain water concentration in Urea-treated mice began to rise and returned to normal at 3 h, but did not return to normal until 6 h post dosing in NaCl-treated mice. Sodium brain concentrations of Urea-injected mice were lower than controls during the first 3 h and were equal to controls at 6 h. No changes occurred in brain K^+ concentrations in Urea-treated mice.

Injection of 120 mmol/kg Urea increased plasma Urea concentration to 150 mM in 15 to 30 min. A prompt clearance of Urea from plasma occurred with a half-life of 2.2 h. A slow, progressive rise in Urea concentration was observed in the brains of mice treated with Urea that peaked at 2 h. The half-life disappearance of Urea from the brain was 4.7 h. Brain and plasma Urea levels were in equilibrium approximately 2.5 h after injection. Urea did not lower plasma glucose. The following parameters were significantly elevated when brain metabolism function was estimated 10 min after treatment with Urea: glucose, lactate, α-ketoglutarate, malate, phosphocreatine, ATP, and energy charge. All metabolic intermediates returned to normal except glycogen (Thurston et al. 1986).

Short-Term Toxicity

Grollman and Grollman (1959) dosed six dogs with bilateral nephrectomies with 5 to 30 g/L of Urea. Urea concentration was maintained in extracellular fluids by peritoneal lavage for 4 to 10 days after study initiation. Toxicity appeared 2 to 4 days after the beginning of dialysis when blood Urea content was 370 to 480 mg%. Signs included weakness, anorexia, vomiting and retching, diarrhea, and a decreased body temperature. Signs gradually increased in intensity and ended with animals in a deep torpor or coma. The concentration of Urea present in blood plasma after 4 to 7 days in the six dogs ranged from 5.4 to 15.6 g/L. Water and electrolyte contents of brain, heart, liver, skeletal muscle, spleen, and gut of nephrectomized animals differed slightly from control animals.

Kornegay et al. (1970) fed 60 Yorkshire pigs several different treatment diets as shown in the third column of Table 8. The basic 9-week feeding trial was designated trial 1A as shown in first column of Table 8. Trial 1A, therefore, included diets 1 to 5.

All diets contained 16% crude protein, were isonitrogenous and isocaloric, and equal in fat, calcium, and phosphorus. The content of protein (nitrogen content minus dietary Urea nitrogen, multiplied by 6.25) for the diets that contained 1% and 2% Urea was 14% and 11.5%, respectively. The pigs were group fed twice daily a rate of 10% of the average body weight raised to the power 0.73. Blood samples were taken one hour after feeding on days 21, 42, and 63.

At the termination of trial 1A, 12 barrows (male pig castrated before sexual maturity) were selected from diets 1, 3, and 5 (4 animals from each diet), referred to as trial 1B. Pigs were individually fed the same diets at the same rate as in trial 1A. Feed concentrations were adjusted weekly, as in trial 1A. The experiment began after an 8-day adjustment period. Urine and feces were collected for 4 days after 3 days on the new feed. This was repeated after an additional 3 days on the new food level. Blood samples were taken at the beginning and end of each experimental period.

In trial 2A, Yorkshire pigs (5/group/sex) were divided into five groups and fed treatment diets for 7 weeks. Methionine and lysine were substituted for an equal weight of corn. All pigs were initially group fed the same level as in trial 1A; however, after 5 to 6 days, pigs receiving diet 5A had not consumed all their feed and the feed level was reduced to 8% of the average body weight raised to the power 0.73. The feed level for pigs that received diet 5 was reduced after 14 days to that of the pigs fed diet 5a. The feed levels were not changed for the remainder of the trial. Blood samples were taken initially and after 16, 34, and 49 days. At the termination of trial 2a, 12 barrows were chosen from diets 1, 3, and 3a. They were fed the same diets and the experimental procedures were the same as in Trial 1B.

In trial 3A, eight groups (5 pigs/sex/group) of Yorkshire pigs were randomly allotted to 10 diet groups. In order to make diet 2 of this trial, corn was substituted for Urea in diet 5 of trial 1A. Amino acids were substituted for an equal amount of corn

TABLE 8
Urea and amino acid supplementation of swine diets (Kornegay et al. 1970)

Trial	Animals/group and duration	Diet	Results/diet group	General results
1A (Feeding trial)	Total of 60 animals; 9 weeks	1: control, corn-soybean meal (SBM)	Weight gain significantly > diets 3, 4, and 5; adequate methionine; food and nitrogen efficiency: 0.38 gain/food and 2.19, respectively	No significant effect of sex or size on gain; average weekly food intake followed same trends as gains; Urea nitrogen values decreased longer pigs were on trial; female pigs had significantly higher hematocrit values in 50% of sampling periods; BUN higher for males—significant in 3/4 of sampling periods
		2: 1% Urea with SBM constant	Weight gain not significant from diet 1; adequate lysine; methionine deficient; food and nitrogen efficiency similar to diet 1	
		3: 2% Urea with SBM constant	Highest BUN value average of 3 intervals, 20.3 mg% vs. 12.2 mg% of control; lysine deficient; decreased food and nitrogen efficiency	
		4: 1% Urea with SBM varied (see text)	Lysine and methionine deficient; decreased food and nitrogen efficiency	
		5: 2% Urea with SBM varied	Weight gain significantly less than pigs of diets 1, 2; significantly lower hematocrit than diets 1, 2, 4; highest BUN value 19.6 mg% vs 12.2 mg% control; deficient methionine and adequate tryptophan; decreased food and nitrogen efficiency	
1B (Metabolism trial)	12 barrows selected from trial 1A; two 3-day intervals	1: same as in trial 1A	BUN was > than diet 1 in feed trial	Body weight gain not significantly affected; Hematocrit values for metabolism diets < those in feeding trial but trends were the same
		3: same as in trial 1A	Nitrogen balance > diets 1 and 5, not significant; hematocrit value < than diet 1; BUN same as feed trial for this group; methionine most deficient in this diet	
		5: same as in trial 1A	BUN same as feed trial for this group; lysine most deficient in this diet	
2A (Feeding trial)	5/group male and female; 7 weeks	1: control (diet 1, 3, and 5 are the same as in trial 1A)	Gained more weight than diets 3, 5, and 5a;	Gain for diets 5 and 5a > diets 3 and 3a; diets 1, 3, and 3a had similar food consumption; serum Urea levels in diets 3, 3a, and 5a returned to control levels as the animals grew older; females had significantly higher hematocrit values 3/4 sampling periods; no sex effect on serum Urea nitrogen concentrations
		3: 2% Urea + SBM constant	Protein efficiency > diet 1; lower hematocrit than control; elevated serum Urea levels at day 16	

(Continued on next page)

TABLE 8
Urea and amino acid supplementation of swine diets (Kornegay et al. 1970) *(Continued)*

Trial	Animals/group and duration	Diet	Results/diet group	General results
		3a: diet 3 + 0.1% DL-methionine	No statistically significant weight gain compared to controls; best protein efficiency; elevated serum Urea levels at day 16	
		5: 2% Urea with SBM varied	Decreased food consumption; same protein efficiency as diet; lower hematocrit value than control; elevated serum Urea levels at day 16	
		5a: diet 5 + 0.5% L-lysine	Decreased food consumption, lower than diet 5; poorest nitrogen and protein efficiency; highest hematocrit value; elevated serum Urea levels at day 16	
2B (Metabolism trial)	12 barrows chosen from diets 1, 3, and 3A of trial 2A; same protocol as trial 1B	1: control (diet 1, 3, and 5 were same as in trial 1A)	Lowest serum BUN	No differences in gain, food consumption and food efficiency between the three diets; hematocrit values were not different between diets
		3: 2% Urea + SBM constant	Increased protein efficiency compared to diet 1; no difference in nitrogen balance from diet 1; intermediate serum BUN	
		3a: diet 3 + 0.1% DL-methionine	Increased protein efficiency compared to diet 1; significant increase in nitrogen efficiency; highest serum BUN	
3A (Feeding trial)	8 groups; 5 pigs/sex/group; 6 weeks	1: positive control (diet 1 of previous trials)	highest food consumption, gain and food, protein and nitrogen efficiency	During group feeding first 3 weeks only control groups had significantly increased gains; the diets fed to pigs in groups 5–10 had significant effects as a result of the tryptophan + methionine and the interaction between lysine and tryptophan + methionine
		2: negative control (diet 5 of previous trials without Urea)	Performance in gain, food consumption and food, protein and nitrogen efficiency was equal to diet 9	
		5: 2% Urea + SBM varied (diet 5 of previous trials)	Significantly lower gain than diet 1	
		6: diet 5 + 0.15% DL-methionine and 0.06% DL-tryptophan	Significantly lower gain than diet 1	
		7: diet 5 + 0.2% L-lysine	Slight improvement in gain, food consumption and food, protein and nitrogen efficiency compared to diet 5	
		8: diet 7 + 0.15% DL-methionine and 0.06% DL-tryptophan	No significant increase in parameters measured in diet 7 with this diet	
		9: diet 5 + 0.5% L-lysine (diet 5a in trial 2A)	Significant decrease in food consumption and decreased gain compared to other diets	

		10: diet 9 + 0.15% DL-methionine and 0.06% DL-tryptophan	Decreased intake of diet 9 overcome by this diet; highest value for protein efficiency	
3B (Metabolism trial)	12 barrows selected from diets 2, 5, and 10 from trial 3A; continued on the same diet and experimental procedures were the same as 1B	2: negative control (diet 5 of previous trials without Urea)	Nitrogen efficiency for other two diets similar to control	Average effects of diet on gain and food and protein efficiency were not different from trial 3A and trend in 3A continued to be present
		5: 2% Urea + SBM varied (diet 5 of previous trials)	Hematocrit and serum Urea nitrogen higher than diet 2, but not significant	
		10: diet 9 + 0.15% DL-methionine and 0.06% DL-tryptophan	Nitrogen balance significantly higher than diet 2; hematocrit and serum Urea nitrogen higher than diet 2, but not significant	
4	48 pigs allotted into 8 groups by sex and litters; 8 weeks	1: basal (10% crude protein corn SBM)	Weekly gain = 2.86 kg; weekly food consumption = 12.72 kg; food, protein, and nitrogen efficiency = 0.22 gain/food, 2.16, and 2.16, respectively	Amino acid imbalance suggested when 0.5% lysine is added to a low protein diet containing Urea because the imbalance effects were not observed in diet 2; non-Urea and Urea-containing diets were not isonitrogenous; significant three-way interaction between Urea, lysine and methionine + tryptophan
		2: basal + 0.5% L-lysine	Weekly gain = 2.97 kg; weekly food consumption = 13.75 kg; food, protein and nitrogen efficiency = 0.22 gain/food, 2.02, and 2.02, respectively	
		3: basal + 0.1% DL-methionine and 0.04% DL-tryptophan	Weekly gain and food consumption better than controls; food, protein, and nitrogen efficiency comparable to controls	
		4: basal + 0.5% L-lysine, 0.1% DL-methionine and 0.04% tryptophan	Food efficiency same as diet 8; greatest nitrogen efficiency	
		5: diet 1 + 1.6% Urea	Weekly gain, food consumption, food and protein efficiency better than controls; nitrogen efficiency < controls	
		6: diet 2 + 1.6% Urea	Decreased food consumption, gain, and efficiency	
		7: diet 3 + 1.6% Urea	Decreased parameters in diet 6 overcome by the addition of tryptophan and methionine	
		8: diet 4 + 1.6% Urea	Decreased parameters in diet 6 overcome by the addition of tryptophan and methionine; greatest weekly gain and food consumption; increased food efficiency	

to make diets 6, 7, 8, 9, and 10. The diets were restriction-fed at the same rate as in the previous trials for the first 3 weeks and then were fed ad libitum for the remaining 3 weeks. Blood samples were not taken in this trial.

Trial 3B consisted of 12 barrows selected from diets 2, 5, and 10 in trial 3A. They were continued on the same diets and the experimental procedures were the same as in trial 1B.

Forty-eight pigs were fed treatment diets ad libitum for 8 weeks in trial 4. Results and a summary of the different diets are presented in Table 8.

In general, the findings supported previous observations that simple-stomached animals, e.g., infants, rats, rabbits, and swine, use some Urea nitrogen; however, the levels of most limiting essential amino acids are extremely important. Additionally, increased blood or serum Urea levels were directly related to the quantity of Urea fed and inversely related to the length of time the pigs were fed Urea. In most cases, the observations made in the feeding trials were confirmed by the results from the metabolism trials (Kornegay et al. 1970).

Balestri (1971) reported a study in which 12 mongrel dogs, with one kidney removed, were injected SC every 8 h with a 10% Urea solution at a dose of 30 to 40 ml/kg for 30 to 45 days. Four dogs were observed for spontaneous movements for 3 days and two dogs had EEG parameters monitored permanently with implanted electrodes. The solution injected was rapidly absorbed, did not cause local side effects and resulted in plasma Urea from 600 to 700 mg/100 ml 20 to 30 min after injection (plasma Urea was 200 to 350 mg/100 ml immediately before the subsequent administration of Urea). Mild drowsiness with reduction in spontaneous movements was the only sign that occurred. The EEG recorded mild changes and no other abnormalities were observed. Necropsy was negative.

Button et al. (1982) dosed orally three 10-week-old littermate male cross-Landrace piglets, designated A, B, and C with 1, 2, and 4 g/kg Urea, respectively. All three pigs were fed an additional 2.5 g% mass/mass (m/m) Urea per day for 5 days. Each piglet consumed the 1 kg of food offered, making the daily intake of Urea approximately 25 g each. As a result, 5% m/m Urea was mixed with the piglet's food for an additional 5 days. One kilogram of meal was offered again and the daily intake was 50 g. Pigs B and C were fed 7.5% m/m and 10% m/m, respectively, for an additional 5 days. The daily intake was 75 and 100 g for pigs B and C, respectively. The palatability of the meal seemed to decrease at this level and the piglets required greater time to consume the 1-kg meal. Piglet A also received single doses of 8 and 16 g/kg Urea orally, with 1 day untreated between all trials.

All three piglets remained healthy during the experiment. No signs of Urea toxicity were observed. Diarrhea was not observed in any of the piglets and their appetites remained good throughout the experiment. Although water intake was not measured, piglet A was observed drinking frequently after the 8 and 16 g/kg doses. All piglets gained approximately 2 kg during the 15-day experiment (Button et al. 1982).

Dermal Irritation

Lashmar et al. (1989) exposed male nude mice to 1%, 10%, 25%, 50%, 75%, or 100% Urea for 24 h. Urea was placed in a polyvinyl chloride cup with a surface area of 0.8 cm^2 and a volume of 0.3 cm^3. The cup was fastened to the dorsal side of the animal using surgical tape and secured with Superglue. Three animals were exposed to each formulation and were maintained three to a cage. The mice were killed immediately after the 24-h exposure period. Specimens of the exposed areas and of adjacent untreated skin were taken for histological evaluation. Histological assessment included examination for epidermal changes, hyperkeratosis, spongiosis, destruction of the epidermis, hyperemia, increase in the cell layers of the stratum granulosum, increase in the density and thickness of the collagen bundles, fractured collagen, infiltration of the dermis, and intracellular edema. Urea did not cause any significant microscopic changes over 24 h at the concentrations tested.

Ocular Irritation

Toxic keratitis was induced in 12 New Zealand white rabbits by administration of 0.5% benzalkonium chloride twice daily for a total of five doses (Charlton et al. 1996). After induction of the keratitis, Urea ophthalmic ointment (2.24%) was administered twice daily for 11 days to the conjunctival sac of each rabbit. Each rabbit received a control preparation, consisting of a mildly hypotonic artificial tear preparation plus the ointment vehicle in the other eye. The rabbits eyes were examined for keratitis and/or corneal epithelial defects every other day for 11 days. All rabbits started the study with an average corneal epithelial defect ranging in size from 30% to 80% of the corneal surface. A statistically significant improvement occurred in the treated eyes compared to the controls. Significant results for intraocular pressure were not observed between treated and control groups. No other extraocular or intraocular changes or abnormalities were noted in either group of rabbits upon completion of this study.

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

In Vitro

Sobhon et al. (1981) incubated rat sperm heads (3×10^8 heads/ml) for 8 h with 8 M Urea and 5 mM dithiothreitol (DTT). Two control experiments were performed; one with whole sperm incubated with 8 M Urea for 8 h and another in which sperm heads were incubated in 8 M Urea and 5 mM DTT for 14 h. Incubation of whole sperm with 8 M Urea caused breaking of the tails from the sperm heads and severe disruption of the tails. The interior of the heads remained uniformly opaque with no chromatin swelling and the sperm achrosomes were almost stripped off by this treatment.

Urea plus DTT treatment for 8 h produced intact and markedly swollen heads. Intact heads had chromatin material within the interior of the sperm nucleus that appeared

electron-dense but the surrounding membranes were almost stripped off. The swollen sperm heads had varying degrees of enlargement and disruption. The disrupted heads had a major portion of the chromatin in the interior of the heads leached off, while the remaining portion had bundles of electron-opaque rods or spherical bodies which may have been cross-sectional profiles of the rods. The chromatin rods were arranged parallel to each other and aggregated along the periphery of the nucleus. In the less disrupted nuclei loosely packed chromatin fibers filled the spaces between the chromatin rods. In the area of the micrograph among the intact and partially broken sperm heads, masses of released chromatin material appeared which consisted of aggregates of chromatin rods and the meshwork of loosely packed chromatin fibers.

The sample incubated with Urea and DTT for 14 h had more swollen sperm heads compared to Urea alone. Loose networks of small chromatin fibers filled the spaces between the chromatin rods regardless of the degree of swelling. A substantial number of heads from this treatment resisted breaking up.

Thin sections of rat sperm heads (3×10^8 heads/ml) incubated for 8 h with 8 M Urea and 5 mM DTT revealed two types of sperm head. One was relatively intact and the other had aggregates of chromatin rods which appear to be derived from the completely broken heads. The interior of the intact heads had thick cords of chromatin running in parallel in alteration with rows of electron-lucent channels depleted of chromatin material interrupted at intervals. The cords and channels run lengthwise along the head-to-tail axis of the sperm nuclei. The Urea-nuclease pellet also had aggregates of dense chromatin rods with a smooth surface (Sobhon et al. 1981).

In Situ

Conner et al. (1976) reported a study of the effectiveness of Urea as a contragestational agent. Female Sprague-Dawley rats were divided into two groups, one treated on day 3 (preimplantation) and the other on day 7 (postimplantation). Each of these groups was further divided into two groups of four to six animals. One group received Urea (0.05 ml) at a concentration of 29% and the other received Urea (0.05 ml) at a concentration of 58% injected directly into one uterine horn. Sodium chloride (0.05 ml at 0.9%) was injected into the other uterine horn as a control. Implantation sites were counted. The number of corpora lutea indicated the expected number of conceptuses in day 3 rats. The animals were killed on day 15 of gestation.

No maternal deaths or toxicity were observed at the doses used in this study. Urea was a very effective contragestational agent at a concentration of 58% injected on day 3 (~1% viable fetuses) and less effective at a concentration of 19% (~40% viable fetuses). The number of viable fetuses in the control site was 80% to 85% for day 3 injections. Neither Urea concentration injected on day 7 was different from the number of viable fetuses in the control site which was between 65% to 80% (Conner et al. 1976).

In Vivo

Topham (1980) studied the effets of 250, 500, 1000, or 2000 mg/kg/day Urea on sperm heads from BALB/c male F_1 mice. The mice received five daily IP injections of vehicle alone or test substance. Sperm were examined for head abnormalities 5 weeks after the last dose. A positive control of 20 mg/kg/day cyclophosphamide monohydrate was included in the experiment. Urea did not significantly increase the incidence of abnormal sperm heads.

Oral

Aleksandrov and Dzhioev (1977) determined that 50 mg/kg Urea did not reduce the teratogenic effects of 30 mg/kg methylurea and 50 mg/kg sodium nitrite in rats dosed orally on the ninth day of pregnancy. Additionally, 50 mg/kg Urea given orally with 10 mg/kg N-nitrosomethylurea (NMU) to rats on the ninth day of pregnancy did not effect the embryonic mortality, frequency or character of the deformities induced by NMU.

Teramoto et al. (1981) orally dosed rats (4) and mice (10) with an aqueous solution of Urea (2000 mg/kg) on days 12 and 10 of gestation, respectively. Control groups received distilled water and were treated in the same manner. Rats and mice were killed on days 20 and 18 of pregnancy, respectively. No observable effect on fetal development occurred in either rats or mice. The mean number of implants, live fetuses, percent fetal resorptions, mean fetal weight, and percent fetuses malformed were comparable to the control group.

Dermal

Inoue and Masuda (1976) dosed ICR-JCL mice with a detergent formulation that contained 17% linear alkylbenzene sulfonate (LAS), 7% ethanol, and 15% Urea as its major ingredients. The detergent was applied dermally (0.5 ml) at concentrations of 0.5%, 5%, and 15% to the shaved dorsal skin (4×4 cm) of pregnant mice once daily per mouse from days 1 to 13 of gestation. The control group received distilled water. The 0.5%, 5%, and 15% groups had 17, 18 and 11 dams, respectively. The control group had 20 dams. Animals were killed on day 17 of gestation.

Maternal body weight gain was not affected by treatment with the detergent. Toxic signs were not observed during the study. The high-dose group had a slightly lower pregnancy rate compared to other treatment groups and the control. Fetal mortality was significantly higher in the high-dose group compared to the control group. A significantly higher placental weight was observed in the 0.5% and 15% groups, although no dose-response relationship was observed. No significant differences among all groups, including the control, were obtained with respect to number of implantations, live fetuses, sex ratio, and body weight of live fetuses. No significant differences in external or internal anomalies were observed in the fetuses between the treated and control groups. Skeletal variations occurred frequently in all groups, including the control. The most frequent

variations included cervical ribs and 14th rib. The incidences of fetuses with 14th rib was significantly low in the 0.5% group (Inoue and Masuda 1976).

Subcutaneous

Inoue and Masuda (1976) used the same detergent formulation as described previously and diluted it with distilled water and dosed mice with 10 ml/kg SC. The mice were dosed once daily at 30 and 150 mg/kg from days 7 to 13 or days 0 to 13 of gestation. Control animals received distilled water. Mice dosed with control, 30 or 150 mg/kg from days 7 to 13 of gestation had 16, 17, and 16 animals/group, respectively. Mice dosed with control, 30 or 150 mg/kg from days 0 to 13 of gestation had 17 animals/group. Mice were killed on day 18 of gestation.

Maternal body weight gain was not affected by treatment with the detergent. No significant changes in general appearance were observed and pregnancy was maintained well in all groups including the control. A significant increase in the number of implantations was observed in the high-dose group days 0 to 13 of gestation. No significant differences were observed between the treated and control groups during the treatment period in the number of implantations or live and dead fetuses, sex ratio, body weight of live fetuses, and placental weight. No significant differences between the treated and control groups were observed for external and internal anomalies in the fetuses. One fetus in the 150 mg/kg group treated during days 0 to 13 of gestation had dislocation of the kidney. Skeletal variations occurred most frequently as a cervical or 14th rib. The incidence of fetuses with extra sternbrae was significantly higher in the 150 mg/kg group treated from days 7 to 13 of gestation than the control group. The incidence of 14th rib in the 30 mg/kg group treated during the same gestation period was highly significant compared to the control. No significant differences for skeletal variations were observed between treated and control groups when the detergent was administered from days 0 to 13 of gestation (Inoue and Masuda 1976).

GENOTOXITY

Effects of Urea on DNA

Ts'o et al. (1962) used calf thymus DNA to study the effects of 3, 4, and 5 M Urea on the ΔTm of polyadenylic acid (poly A) and thymus DNA. Most nucleic acids and synthetic polynucleotides have a helix-coil transition within a narrowly defined temperature zone (*Tm*) at a given ionic strength and pH. The *Tm* may be affected by the interaction of bases or nucleosides with a nucleic acid and, as a result, the nucleosides may be bound preferentially to the coil form of the polymer and hinder the formation of the helix. *Tm* was defined as the temperature at the midpoint of the helix-coil transition, measured optically. ΔTm was defined as the lowering of *Tm* at a constant pH and ionic strength as a result of an interacting substance. The concentration of poly A and DNA were 1 to 1.4 mg/ml which gave an optical rotation of 0.4 to 0.6 for poly A and 436 mμ for DNA. The experimental ΔTm for poly A obtained at the maximum concentration of Urea used was $2^{\circ}C \pm 1^{\circ}C$. The experimental ΔTm for thymus DNA was $15^{\circ}C \pm 1^{\circ}C$ at 5 M Urea.

Chang and Li (1974) used a Urea treatment in a study of nucleohistones. Soluble and NaCl-treated, partially dehistonized nucleohistones were prepared from calf thymus chromatin. The nucleohistone preparation was exposed to 5 and 8 M Urea in an EDTA buffer. The fraction of histone bound base pairs was 78% for native nucleohistone, 60% for 0.6 M NaCl-treated, and 34% for 1.6 M NaCl-treated nucleohistone.

The derivative melting curve of native and NaCl-treated partially dehistonized nucleohistones from calf thymus in EDTA was distinguished as three melting bands and a shoulder. Melting band I at $47^{\circ}C$ corresponded to the melting of free base pairs and band II, the shoulder of the curve, at $57^{\circ}C$ corresponded to those free base pairs bound by nonhistone proteins or small gaps between histone-bound regions. Bands III at $72^{\circ}C$ and IV at $82^{\circ}C$ corresponded to those free base pairs bound by histones. In general, the addition of Urea to native nucleohistone lowered the melting temperatures of these melting bands in the order of I > II > III > IV. At higher Urea concentrations a new melting band, V, occurs at $95^{\circ}C$ to $100^{\circ}C$.

Similar results were reported for the addition of Urea to 0.6 M NaCl-treated nucleohistone. Additionally, no full or partial dissociation of native histones from DNA could be detected from either 5 M Urea or from 0.6 and 1.6 M NaCl treated nucleohistone at various concentrations.

The circular dichroism (CD) of native nucleohistone in the absence and presence of 5 M Urea was also studied. Urea increased the positive CD band at 275 nm and reduced the negative band at 220 nm. The reduction of the negative band was interpreted as a destruction of α-helix of bound histone by Urea.

Similar results were observed for the effect on the CD spectrum of 0.6 M NaCl-treated nucleohistone. The CD spectrum of 1.6 M NaCl-treated nucleohistone was different from native and 0.6 M NaCl-treated nucleohistone. Although 5 M Urea reduced the negative CD at 220 nm, the positive CD band with 1.6 M NaCl-treated nucleohistone was reduced. The $\Delta\epsilon_{220}$ ($\Delta\epsilon$ was defined as the difference between molar extinction coefficient for the left- and right-handed circularly polarized light) at higher concentrations of Urea became less negative for native and partially dehistonized nucleohistones. The reduction was proportional to the Urea concentration. The CD at 220 nm for pure DNA did not change in the presence of Urea, but at 275 nm it was reduced. No change at $\Delta\epsilon_{278}$ occurred at less than 2 M Urea for native and 0.6 M NaCl-treated nucleohistones.

At concentrations greater than 2 M an increase was observed at this wavelength. A decrease of $\Delta\epsilon_{278}$ up to 3 M Urea occurred in 1.6 M NaCl-treated nucleohistones. Beyond 3 M Urea, this trend is reversed. The original structures of native and NaCl-treated partially dehistonized nucleohistones can be mostly restored after Urea perturbation (Chang and Li 1974).

Nielsen et al. (1985) studied the effects of Urea on chromatin which contained all histones (H1, H2A, H2B, H3, and H4) isolated from mouse Ehrlich ascites tumor cells. Flow linear dichroism measurements were performed on a Jasco J-500 spectropolarimeter at a flow gradient of 1800 s^{-1}. Photoaffinity labeling of chromatin was performed using the ^{3}H-labeled arylazido-bisacridine *N*, *N*′-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diamineooctane which labeled H1, H2A, and H3. Chromatin, corresponding to 100 μg DNA, plus 0 to 7 M Urea was added to the labeling reagent. This mixture was irradiated with a pyrex-filtered light from an Osram Sp 200 superpressure mercury lamp for 10 min (100 kJ/m^2) while in an ice bath. The histones were then extracted. Nuclease digestion studies were performed using 1 U micrococcal nuclease for 34 μg chromatin.

In the absence of Urea, the LD_r (reduced linear dichroism) is slightly positive; however, in the presence of Urea the LD_r became negative and increased in magnitude with increased Urea concentrations. Urea-induced uncoiling of the DNA from nucleosomal supercoils is accomplished through two transitions, the first one occurred between 1 and 2 M Urea and the second between 6 and 8 M Urea. The smoothly changing circular dichroic signals indicated that no corresponding conformational distinct transitions occurred in the histones or in the structure of the DNA helix. The LD_r is almost constant within 2 to 6 M Urea. At 8 M Urea, the LD_r was about 60% of the value of chromatin stripped of histones in 2 M NaCl.

The presence of Urea caused significant changes in the photoaffinity labeling of histones. The labeling of the linker histone, H1, increased until 2 M Urea and was constant at 2 to 5 M Urea. The core histones, H2A and H3, had a peak at 4 M Urea, whereas the core histone H2B is labeled only above 3 M and the labeling of H4 was almost not observed. Under similar experimental conditions, the labeling of histones in the absence of DNA only labeled H3, regardless of the Urea concentration and a decrease in labeling efficiency was observed at increasing Urea concentrations. As Urea concentration increased to 2 M, the flexibility of the linker DNA increased, allowing for a wider distribution of the intercalation sites in the linker and exposure of more photoreaction sites on the histone which resulted in the increased labeling of H1, H2A, and H3. The core histones are more accessible to the labeling reagent between 2 and 4 M Urea.

These authors also observed an increase in the susceptibility of the linker DNA to nuclease digestion between 1 and 3 M Urea, while digestion was almost completely inhibited above 4 to 5 M Urea. No change in the size of DNA fragments produced was observed in the presence of Urea. The activity of the nuclease only had a 50% inhibition in 7 M Urea (Nielsen et al. 1985).

Genotoxicity Assays

A summary of genotoxicity assays with Urea is presented in Table 9. Urea was not genotoxic in several bacterial and mammalian assays. In assays where Urea was used at a high concentration, genotoxicity was found, many in in vitro assays.

CARCINOGENICITY

Shear and Leiter (1941) injected Urea into 20 strain A and 10 C57 black male mice. The initial dose was 10 mg and was progressively increased to 50 mg. Repeated injections were given over an 11-month period until a total of 800 mg was given to the mice. Of the 19 mice that lived 1 year, 5 survivors were killed after 15 months. No treatment-related tumors were observed.

Fleischman et al. (1980) fed Fischer 344 rats and C57B1/6 mice (50/sex/group/species) ad libitum with diets containing 0.45% (low), 0.90% (medium), and 4.5% (high) Urea for 365 consecutive days. The control diet did not contain Urea. All treated and control animals were placed on study over a period of 2.5 months for rats and 13 months for mice. After 365 days of treatment 5 animals/sex/species from control and test groups were killed and examined. The remaining survivors were placed on a control diet for 4 months and necropsied at the end of this time period.

No weight depression for animals of either sex or species was observed at terminal necropsy. Male rats of the middle test dose (0.90%) had decreased survival (89%) compared to controls (95%). Survival of all other test groups was not affected. A significant linear trend and an increased proportion of interstitial adenomas of the testis was observed in the high dose group of rats.

A non–dose-related increase in the occurrence of malignant lymphomas was observed in female mice treated with mid-dose level of Urea. Morphological features included lymphoid architecture effaced by sheets of pleomorphic cells with large vesicular oval, round, elongated, and irregularly shaped nuclei with prominent nucleoli and moderate amounts of pink cytoplasm, as well as occasional giant cells and areas of karyorrhexis and karyolysis.

Because no significant increase in lymphomas occurred in the low- or high-dose groups and because the interstitial cell adenomas of the testes could occur in 100% of the controls, the investigators considered Urea to be a noncarcinogen (Fleischman et al. 1980).

Cocarcinogenicity

Mann et al. (1991) established a rat urinary bladder carcinogenesis model in vitro. Primary rat bladder epithelial cells were grown in the presence of 2-amino-4-(5-nitro-2-furyl)thiasole (ANFT) for 4 weeks followed by a long-term exposure to Urea, sodium saccharin, or control medium. Another set of cultures was exposed to ANFT, Urea, and sodium saccharin simultaneously. A summary of treatments is presented in Table 10.

Anchorage independence was estimated by growth in soft agar. All treated cells except one treated with sodium saccharin were anchorage independent. Cells ($1–3 \times 10^6$ cells) from the treated cultures were innoculated into athymic C57/BALB/c

TABLE 9
Urea genotoxicity assays

Assay/test system	Dose	Protocol	Result	Reference
		In Vitro		
Bacteriophage				
T4 DNA-transforming activity	8 M Urea with or without 5 min heating	Treated bacteriophage T4r$^+$ DNA incubated with spheroblasts and π factor, heated, and plated on *E. coli* K-12(λ) and *E. coli* B to measure r^+ transformants.	8 M Urea in combination with 5 min heating at 55°C increased the number of transformants by a factor of 3–4 and at 65°C by a factor of 5–6; 8 M Urea alone caused no increase	Gol'dfarb and Sergeeva 1968
Bacterial cells				
Lysogenic induction	0.1–3.2 M	*Corynebacterium diptheriae* strain 25 and C7; repeated 5x; pH 4.2, 5.0, or 7.2	No evidence of genotoxicity	Kozak and Dobrzański 1970
Ames test	Not stated	*S. typhimurium* TA 98, 100, 1537; ± metabolic activation; preincubation 20 min prior to cell plating	No evidence of genotoxicity	Ishidate and Yoshikawa 1980
RK bacterial test	1.0 mg/ml	*E. coli* CHY832; 0.20 ml Urea added to suspension incubated for 10 min at 30°C; cell culture incubated for 48 h at 42°C; ± metabolic activation	No evidence of genotoxicity	Hayes et al. 1984
Ames test	5, 10, 50, 100, 500, 1000, 5000 μg/plate	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* (WP2uvrA); ± S9	No evidence of genotoxicity	Shimizu et al. 1985
Amphibian cells				
Frog lymphocyte culture	5%	Cells in G_o exposed to Urea for 15 and 30 min. then x-irradiated: 200 Roentgen (R) total dose; washed 3 times; unirradiated and untreated lymphocytes and lymphocytes treated with 5% Urea for 15 min were controls	5% Urea not mutagenic 200 R, 15 min Urea, 31% aberrations; 200 R, 30 min Urea, 40.8% aberrations; 200 R 42% aberrations; control and 5% Urea, 0.1 and 0.2% aberrations, respectively	Pankova and Sokolov 1974
Mammalian cells				
Human lymphocyte culture	0.01, 0.1, 1.0 mg/ml	2–5 repetitions were conducted for each dose; 100–400 metaphase were analyzed at each repetition	No evidence of genotoxicity	Zhurkov 1975
Mouse lymphoma L5178Y assay	0, 0.132, 0.265, 0.397, 0.530, 0.662 M	No metabolic activation; heterozygous L5178Y TK$^{+/-}$ −3.7.2C cells; pH 7.2; exposed for 4 h	Only positive at concentrations that reduced cell survival below acceptable levels[a]	Wangenheim and Bolcsfoldi 1988
Mouse lymphoma L5178Y/TK$^{+/-}$	0, 0.359, 0.449, 0.539, 0.628, 0.718 mole/L	3-H exposure; −S9	Positive at high concentrations only (0.628 M and above; 4% cell survival)[a]	Garberg et al. 1988
Mouse lymphoma L5178Y/TK$^{+/-}$/DNA alkaline unwinding test	0, 0.359, 0.449, 0.539, 0.628, 0.718 mole/L	Samples maintained at pH 6.8; −S9	Positive at high concentrations only (0.628 M and above; 4% cell survival)[a]	Garberg et al. 1988

Normal human leukocytes	1, 50 mM	Incubated 72 h	Negative at 1 mM; positive at 50 mM (severe chromosome fragmentation and moderate cell damage)	Oppenheim and Fishbein 1965
Chromosome aberrations in Chinese hamster fibroblasts of lung origin (CHL)	Various concentrations of Urea and sodium chloride	10^5 CHL cells in monolayer culture; exposed to 50% inhibition dose; harvested 24 and 48 h after treatment; ±S9	13.0 mg/ml was the Urea concentration at which chromosome aberrations were detected in 20% of metaphase cells; there were 0.06 exchange type aberrations per mg/ml Urea concentration; sodium chloride produced more chromosome aberrations than did Urea	Ishidate et al. 1981
Chromosome aberration (CHL)	16.0×10^{-4} M (max. effective dose mg/ml)	10^5 CHL cells in monolayer culture; harvested 24, 48, and sometimes 6 h after treatment	Positive only at 2.7 M 16% solution	Ishidate and Odashima 1977
Metabolic cooperation/T2-14 clone of Chinese hamster V79 cells	0–5 mg/ml	200 T2-14 cells + 1.5×10^5 wild-type cells incubated 1 day; 6-thioguanine plus Urea added + 0.001–1.0 μg/ml 12-*O*- tetradecanol phorbol-13-acetate	moderate increase in the recovery of T2-14 cells; toxic effects observed at concentrations close to max effect (~5 mg/ml)	Umeda et al. 1980
		In Vivo		
Animal				
Sperm head abnormality test	250, 500, 1000, 2000 mg/kg/day	5 male mice/group (CBAxBALB); 5 daily IP injections; 5 weeks after last dose, caudal sperm examined	No evidence of genotoxicity	Topham 1980
Chromosome aberration (bone marrow cells)	500 mg/day	Albino Swiss mice were fed 500 mg/day Urea for 5 days and sacrificed 7 days after treatment termination	Chromosome aberrations found	Chaurasia and Sinha 1987
Plant				
Chromosome aberration *Allium cepa* (onion)	2%	Bulbs were grown for 24 h over a 2% aqueous solution of Urea	Chromosome aberrations found	Chaurasia and Sinha 1987
Meiotic anomalies in *Allium cepa*	2.5, 5%	*A. cepa* L. buds were sprayed with test concentrations of Urea and exposed for 24 and 48 h; controls were sprayed with distilled water	Anomalies found at all meiotic phases, especially metaphases I and II; most common anomaly was chromosome clumping	Chaurasia and Rathore 1976
Chromosome aberration *(Vicia faba)*	2.5×10^{-1} moles	Urea pretreatment and simultaneous treatment with x-rays, [^{3}H]Thymidine, ethyl alcohol, mitomycin C, maleic hydrazide	No evidence of genotoxicity	Schubert and Rieger 1976

[a]Doses that produce excessive toxicity (low cell survival) typically yield spurious results.

TABLE 10
Summary of rat urinary bladder carcinogenesis model treatment groups (Mann et al. 1991)

Group	Initial treatment (4 weeks)[a]	Second treatment (weeks)[a]	No. of mice with tumors/injection
1, 2	ANFT	NaS, Urea (28)	2/5, 6/6
3	ANFT + NaS + Urea	ANFT + NaS + Urea (20)	3/5
4	ANFT + NaS + Urea	ANFT + NaS + Urea (29)	3/5
5, 6	DMSO	DMSO (20)	nd[b], 0/5
7	ANFT	DMSO (24)	1/5
8	ANFT	DMSO (20)	5/5
9, 10	ANFT	NaS (20)	0/7, 0/2
11	DMSO	NaS (33)	0/2
12	DMSO	NaS (22)	0/3

[a]2-Amino-4-(5-nitro-2-furyl)thiazole (ANFT) at 1 μg/ml; sodium sulfide (NaS) at 25 μg/ml; Urea at 0.05%; dimethylsulfoxide (DMSO) at 0.05%.
[b]Not determined; cell line died out; did not recover from cryopreservation.

female nude mice on either side subcutaneously. Control mice received cells from experiment 6 (see Table 10). Mice were examined twice weekly for signs of tumor formation. The tumor was considered negative for transplantability if no tumor appeared within 6 months of inoculation.

The cells in the treated group were able to grow off the collagen and onto plastic, an indication of transformation, within 5 to 7 months. Groups 5 and 6 took 4 and 12 months, respectively, to grow on plastic. After 14 days growth in soft agar, anchorage dependence was observed in groups 1, 2, 3, 4, 7, 8, 9, 10, and 12. Anchorage independence was not determined in group 5, because the cell line died out and did not recover from cryopreservation; no anchorage results were presented for groups 6 and 11.

The number of mice with tumors/injected was, respectively: groups 1 and 2, 2/5 and 6/6; groups 3 and 4, 3/5 each; group 5, not determined and group 6, 0/5; groups 7 and 8, 1/5 and 5/5, and groups 9, 10, 11 and 12, 0 each. Tumor latency was, respectively: groups 1 and 2, 9 to 10 and 4 to 7 weeks; groups 3 and 4, 7 to 10 and 12 weeks; groups 5 and 6, information not available; groups 7 and 8, 16 and 3 to 4 weeks; groups 9 and 10, 0 weeks each, and groups 11 and 12, information was not available. Microscopic evaluation determined the following tumor types in each group, respectively: groups 1 and 2, poorly differentiated transitional cell carcinoma (TCC) and squamous cell carcinoma (SCC); group 3, SCC and TCC; group 4, SCC; groups 7 and 8, adenocarcinoma and TCC with adenocarcinoma in the same tumor. Tumor types were not available for groups 5, 6, 9, 10, 11, and 12. Staining for cytokeratin was positive in groups 1, 2, 4, 7, and 8 and negative in group 3. Information on cytokeratin staining was not available for groups 5, 6, 9, 10, 11, and 12 (Mann et al. 1991).

CLINICAL ASSESSMENT OF SAFETY

Eknoyan et al. (1969) conducted a test in which 10 normal volunteers ingested 2 to 3 g/kg Urea at hourly intervals. Serum Urea nitrogen levels of 60 to 120 mg% were maintained for 8, 10, or 24 h. Each volunteer served as their own control. No variation was observed for platelet counts; however, platelet adhesiveness was reduced. Platelet adhesiveness was also significantly reduced in 15/26 patients with renal failure when serum Urea nitrogen increased to a level $\geq$100 mg%.

Bensinger et al. (1972) reported on eight black volunteers with sickle cell disease who ingested 8 to 40 g of Urea two to five times per day for at least 3 weeks. Urea had no statistically significant effect on red blood cell survival in these volunteers.

Wolfe et al. (1982) studied four healthy male volunteers who received di-[^{15}N]Urea at an infusion rate of 18.9 μmol/min with a priming dose-infusion ratio of 300:1 at a rate of 0.191 ml/min. The subjects received the isotope while at rest and during exercise on an ergometer that averaged an intensity of 360 kpm/min (kpm not defined). Heart rate remained at 110 beats/min and was maintained at this rate for 105 min. Neither Urea concentration nor Urea production was affected by exercise. The net protein catabolism calculated from Urea data at rest and during exercise was 0.042 ± 0.003 g/kg/h.

Dermal Irritation

Johnson et al. (1970) applied saline solution, Urea, and autogenous urine specimens to the arm or forearm of six healthy male volunteers using the skin window technique. The sodium chloride, Urea, and autogenous urine solutions were prepared as a hypotonic, hypertonic, or isotonic solution. Three skin windows were made on each of the six volunteers. One drop of the saline solutions was placed in each of the three skin windows of two of the volunteers (one window was used for each concentration) 7 h after the skin window was established. One drop of the Urea solutions was placed in the same way in the three skin windows of the second two volunteers. One drop of each of the three diluted autogenous urine specimens was placed in the skin windows of the remaining two volunteers. The respective

solutions were added to the skin windows at 24 h with one drop of India ink to evaluate the phagocytic ability of the cells in the exudate. Cover slips were changed at 10 h, evaluated, and removed at 28 h and reevaluated.

Skin windows to which the isotonic saline solution had been added showed no abnormalities in the number, type or structural integrity of the responding cells. The exudate in the windows to which the hypertonic saline solution had been added had a marked diminution in the number of cells responding. The mononuclear cells appeared to be normal in quality but decreased in quantity. The multinuclear cells were decreased and had destructive changes. Skin windows to which hypotonic saline had been applied had marked depletion of cells especially in the multinuclear series. The mononuclear cells that responded were swollen and vacuolizated. Studies for phagocytosis with saline had only minor variations from the normal response. Studies with isotonic Urea had both normal and abnormal results. Hypertonic Urea decreased the number of responding cells in both subjects and toxic changes were observed with the mononuclear cells affected more than the multinuclear cells. Hypotonic Urea produced slight to moderate diminution in the mononuclear series with moderate to marked cell disintegration and destruction. In general, studies that evaluated phagocytosis in the presence of Urea reported a mild to marked decrease in phagocytosis. A marked depletion of cells, regardless of toxicity, occurred in all instances in which autogenous urine was added to the skin windows. The exudate induced by isotonic urine appeared to consist of multinuclear cells. Cells responding to hypertonic urine were mononuclear with marked vacuolization and budding. A diminished response with cell destruction also occurred in hypotonic saline exposure. All skin windows exposed to urine showed a failure of phagocytosis associated with extensive cellular destruction (Johnson et al. 1970).

Hill Top Research (1977) applied a lotion containing 5% Urea via a patch to the backs of 10 panelists for 21 consecutive days or until maximum irritation was observed. Approximately 0.3 ml of the sample was applied to the test patch. The sample was reapplied to the same test site 23 h after application. Reactions to the test samples were scored by a staff member 24 h after application (1 h after patch removal). The lotion containing 5% Urea was slightly irritating.

Fair and Krum (1979) conducted a study in which 16 male subjects received daily applications of 0.3 g of each of the following test materials in a closed patch fashion: 1% hydrocortisone in a "specially formulated" (polysaccharide-stabilized) 10% Urea base; the base alone; hydrocortisone acetate 1% in a regular 10% Urea base; 20% Urea cream with nonlipid emollients and white petrolatum. The substances were applied for 21 days to a paraspinal location and irritation evaluations were performed 30 minutes after the end of each 24-h period. None of the test materials demonstrated irritation, except the 20% Urea cream, which was significantly more irritating than the other test materials, because all subjects were irritated by this test material. Five subjects exhibited irritation to the 1% hydrocortisone acetate regular 10% Urea base whereas no subjects exhibited irritation to the 1% hydrocortisone in a 10% stabilized Urea base.

Gollhausen and Kligman (1985) applied Urea (60%) in a chamber to the forearm of four subjects for 3 days. Pressure was applied to the treated skin areas by placing a 9 mm diameter wood bead atop the chamber and tightly encircling the forearm with adhesive tape. No significant reaction was observed in subjects 30 min after removal of the chamber.

Agner (1992) conducted a study in which 17 healthy volunteers (13 male and 4 female subjects) participated to determine the irritant effects of Urea in different vehicles. The flexor side of the upper arm was used as the test region in closed patch tests which used Finn test chambers. A test concentration of 20% Urea in water or petrolatum was used during the study. Test chambers with the vehicles served as controls. Test chambers were removed after 24 h. Patch test sites were evaluated prior to patch testing and 60 min after 24-h and 48-h exposures. Visual scoring, cutaneous blood flow, evaluation of skin thickness and transepidermal water loss (TEWL) were evaluated.

The visible reaction to the Urea/petrolatum patches was more significant than the reactions to the Urea/water patches. A significant increase in cutaneous blood flow occurred in patches with Urea/petrolatum after 24 h, compared to pre-values and the petrolatum control; however, blood flow was normalized after 48 h. No increase in blood flow was observed when water was the vehicle for Urea; however, a significant increase in blood flow occurred in water patches after 24 h compared to pre-values. Cutaneous edema was observed after 24 h exposure to Urea/petrolatum patches and skin thickness was increased significantly from the pre-value and the petrolatum control. Urea/water patches also had a significant increase in skin thickness after 24 h compared to pre-values, but not when compared to the water control value. A significant increase in skin thickness occurred in water patches compared to pre-values after 24 h. TEWL was significantly increased in patch test areas exposed to Urea/petrolatum for 24 h compared to pre-values and the petrolatum control. TEWL values were normalized after 48 h. A significant increase was not observed for the Urea/water TEWL value as compared to the pre-value and water control (Agner 1992).

Serup (1992) treated healthy volunteers with a 3% (22 volunteers) or 10% (23 volunteers) Urea cream in a double-blind study. The 3% Urea cream also had the following ingredients: 1.5% lactic acid, 1.5% betaine, propylene glycol, mineral oil, polyethylene glycol-5 stearyl stearate, ethylhexyl ethylhexonate, steareth-21, cetearyl alcohol, self-emulsifying glyceryl stearate, thomethamine, fragrance, water, 14% lipid content, and a pH of 3.5. The 10% Urea cream also had the following ingredients: 5% lactic acid, betaine, propylene glycol, cetearyl alcohol, ethyhexyl ethylhexanoate, tromethamine, self-emulsifying glyceryl stearate, diethanolaminecetyl phosphate, dimethicon, fragrance, water, a lipid content of 6%, and a pH of 3.5. The flexor side of the forearm was treated with two applications of the test cream daily for 3 weeks. The contralateral side was the

untreated control. The dose of the lotion was left to the users' preference in order to create a realistic situation. The mean concentration of test cream used per application was 0.021 g/cm^2. The last application occurred 12 h before the measured response. A blinded clinical evaluation was performed and skin color, electrical capacitance which indicated hydration of the outer epidermis and skin surface and TEWL were measured instrumentally. Scaling and epidermal desquamation were assessed using the tape strip method.

The amelioration of dry skin was the same between the two treatment groups. The 3% Urea cream was cosmetically more acceptable because it left the skin softer and more pliable, whereas the 10% Urea cream left a sticky film on the skin surface. Stinging occurred in three cases with the 10% Urea cream. No skin irritation was reported by the volunteers or observed by the dermatologist's evaluation. Blind clinical evaluation by a dermatologist reported that the treated skin surface had increased stickiness. Color measurements reported no evidence of inflammatory skin reactions or irritation. The electrical capacitance and conductance was significantly increased in all groups. TEWL was not significantly changed in the 3% Urea group, but was decreased significantly in the 10% Urea group. Optical transmission through tape increased in all groups and no differences were observed between the 3% and 10% groups. Blind visual assessment of the tapes reported reduced scaling scores for both concentrations of Urea creams (Serup, 1992).

Treffel and Gabard (1995) exposed two groups of healthy volunteers to two oil-in-water emulsions (a cream and lotion that contained 2% Urea) and two water-in-oil emulsions (a fatty cream and a lotion that contained 4% Urea). The first group had three men and three women and the second group had six men. The emulsion formulations were applied (2 mg/cm^2) to the forearm and gently rubbed in with a gloved finger. The remaining material was wiped off 1 h later and tests were conducted 5 min later on each of the treated sites. The contralateral forearm served as the control site.

Sorption-desorption tests had the highest values after treatment with the lotions that contained Urea. Hygroscopicity had a tendency to decrease after treatment with the moisturizers that contained Urea as significant results occurred with the oil-in-water creams. The water-holding capacity was significantly increased after treatment with the oil-in-water creams and lotions (2% Urea). No statistically significant results were obtained for both lotions with respect to the water accumulation test. Control test results indicated intra- and interindividual variability for prehydration states, hygroscopicity, water accumulation velocity, water-holding capacity, and water accumulation (Treffel and Gabard 1995).

TKL, Inc. (1997) evaluated a body cream containing 3% Urea to determine its ability to sensitize the skin of normal volunteers using an occlusive Finn Chamber repeat-insult patch test (RIPT). Fifty subjects completed the study. There was no evidence of sensitization or significant irritation to the body cream containing 3% Urea.

Zhai et al. (1998) evaluated the skin-protective properties of beeswax, paraffin wax, a derivative of lanolin and cetyl alcohol against the irritant 22.9% ammonium hydroxide/2.0% Urea formulation in water in 10 healthy, male Caucasian volunteers. The test material (0.05 ml) was applied to a 2.0-cm^2 area of the forearm and allowed to dry for 30 min before a second application. The test material was applied under occlusive patch conditions. The patches were removed after 24 h and scoring occurred 72 h post application. Cutaneous reactions were scored on a 10-point scale. Ammonium hydroxide plus Urea in paraffin wax plus beeswax in cetyl alcohol (score = 3.87) and the lanolin derivative in cetyl alcohol (score = 4.02) significantly reduced irritation. The untreated control site had a score of 5.42.

A 3% Urea formulation was tested by the Consumer Product Testing Co. (1999) for dermal irritation/sensitization in an RIPT consisting of 214 subjects. Under the conditions of this study, the test material did not indicate a potential for dermal irritation or sensitization.

Lodén et al. (1999) studied 15 patients of both sexes with rough or clinically normal skin on the volar aspect of the forearm and eczema on other parts of the body to evaluate the skin barrier function of a cream that contained 5% Urea in an oil-in-water emulsion at a pH of 5. The cream also contained about 19 other ingredients. The volar aspect of one of the forearms was treated twice daily for 20 days with the cream. TEWL and skin capacitance were recorded at the start of the study, and after 10 and 20 days of treatment. The following day, treated and untreated skin areas were exposed to 14% sodium lauryl sulfate (SLS) for 7 h and the resultant irritant reaction was assessed.

The TEWL was lower on the treated site than the untreated site. However, skin capacitance increased significantly compared to the untreated site. After exposure to SLS, visual assessment reported fewer of the sites treated with Urea showing irritation than the untreated sites, (40% versus 67%) with mean irritancy scores of 0.7 and 1.1, respectively. TEWL and superficial skin blood flow had significantly lower values in the treated site compared with the untreated site. The investigators stated that it was possible that the canola oil in the cream may have affected the results (Lodén et al. 1999).

Penetration Enhancement

Feldmann et al. (1974) applied a Urea-containing cream and a cream without Urea to the forearm of five normal male subjects. The cream consisted of 1% hydrocortisone acetate, 10% Urea, 23% emollients (stearic acid, isopropyl myristate, isopropyl palpitate, polyalkyloxy fatty acid ester, and acetyl alcohol), 5% other pharmaceutical aids (carboxy vinyl polymer, propylene glycol, xanthan gum, triethanolamine stearate, sodium lauryl sulfate, sodium bisulfite, edentate disodium, and perfume), and 61% water. The cream had radiolabeled and unlabelled hydrocortisone acetate present. In the control cream tested, Urea was omitted and the water content increased to 71% so that the other

ingredients would be at the same concentration as in the 10% Urea cream. The subjects were dosed with 100 mg and dose sites remained unoccluded. The area of the dose site was 66 cm^2. All urine was collected from each subject for 10 days, the first day in three 4-h periods and then a 12-h period. The control cream was studied on the same subjects 3 weeks after the test cream. The amount of hydrocortisone absorbed with the Urea containing cream was 1.02% compared to the control's 0.49%. The doubling of absorbed hydrocortisone with Urea was considered statistically significant.

Wohlrab (1984) exposed human skin from the inguinal region in vitro to the following preparations: 1% hydrocortisone without emulsion, 1% and 0.5% hydrocortisone plus 10% Urea, 0.1% triamcinolone acetonide without emulsion, and 0.1% and 0.05% triamcinolone acetonide without emulsion plus 10% Urea adsorbed onto particles of polysaccharide. Approximately 16 mg of ^{3}H-labeled corticosteroid ointment was applied to the test area of 4 cm^2. The penetration period was measured at 10, 30, 100, 300, and 1000 min.

The addition of 10% Urea resulted in a two- to threefold increase in the concentration of hydrocortisone within the horny layer, epidermis, and dermis. The increase observed after longer periods of penetration into the deeper layers of the dermis is due to a retention of the substrate which is frequently seen under these conditions in vitro. The concentration of hydrocortisone in the dermis and epidermis after treatment with 1% hydrocortisone at 1000 min was 0.24 and 0.15 $\mu g/cm^2$, respectively. Treatment with 0.5% hydrocortisone plus 10% Urea yielded concentrations of 0.26 and 0.54 $\mu g/cm^2$ for the epidermis and dermis, respectively, at the same time point. Similar results were obtained with triamcinolone acetonide, where the concentration was increased in all three layers studied; however, treatment with 0.05% triamcinolone acetonide plus 10% Urea resulted in similar concentrations as those reported with creams containing twice the steroid content but without Urea (Wohlrab 1984).

Krochmal et al. (1989) used skin samples from the abdomen or chest of human cadavers to study the in vitro skin penetration of various Urea preparations. Urea (10%) was compounded with hydrocortisone 17-valerate, desoximetasone, triamcinolone acetonide and fluocinonide. A 50-mm^3 sample of the cream was applied to the skin section. The receptor fluid was collected at 1, 2, 3, 4, 7, 8, 12, 24, 32, and 48 h. Physical stability studies were also evaluated at ambient conditions before compounding and at 24 h, 1 month, and 2 months after compounding.

The penetration of hydrocortisone 17-valerate through human skin was not affected by 10% Urea. The penetration of desoximetasone through human skin was not statistically significant when compounded with 10% Urea. However, the penetration of triamcinolone acetonide with 10% Urea was significantly higher than the cream alone. Fluocinonide cream plus 10% Urea did not penetrate the skin after 72 h and the investigators suggested that chemical instability contributed to this occurrence.

The decrease in steroid content after about 2 months was 10% for hydrocortisone 17-valerate, 20% for desoximetasone, 45% for triamcinolone acetonide, and 34% for fluocinonide. The steroid content decreased more than 10% immediately after it was mixed with Urea, which suggested that chemical degradation had occurred. Due to a possible vehicle-adjuvant interaction, the pH of triamcinolone acetonide cream plus 10% Urea was higher than the other preparations. The pH of most preparations was unchanged at ambient conditions for 2 months (Krochmal et al. 1989).

Lippold and Hackemüller (1990) studied the effect of a 10% Urea solution (pH 6.1 to 6.3) on the penetration of benzyl nicotinate in 10 volunteers. Each volunteer submerged one forearm in a moisturizer solution (10% Urea) and the other in deionized water for 20 min. The arms were allowed to dry for 30 min. Benzyl nicotinate ointment in petrolatum at concentrations from 10^{-3} *w/v* and 0.313% to 3% were applied to circular areas 15 mm in diameter to the inner side of each forearm. The ointment applied was spread out into a layer of ointment of 28 μm. Urea visibly crystallized on the skin after drying which followed the pretreatment and hindered the observations for redness. Urea had no significant influence on the penetration of benzyl nicotinate.

Sznitowska et al. (1996) conducted an in vitro study in which human cadaver full-thickness skin was used to determine the penetration enhancement effect of 10% Urea on saturated solutions of the zwitterion baclofen. The penetration area was 0.64 cm^2. After a 6 to 8-h equilibration period, 300 μl of the donor solution (5 mg/ml baclofen plus 10% Urea) was placed onto the skin. The receiver solution was changed every 12 h for 60 h and the donor solution was changed every 24 h. Urea did not increase baclofen solubility; however it did increase the lag time to 24 h compared to the <6 h for the water vehicle. No significant observations between control and treated skin samples occurred with respect to steady-state flux or the permeability coefficient. The amount of baclofen in the epidermis and dermis after 60 h and treatment with 10% Urea was 6.9 ± 1.5 $\mu g/cm^2$ and 5.9 ± 1.6 $\mu g/cm^2$, respectively. The control values for epidermis and dermis were 6.5 ± 2.3 $\mu g/cm^2$ and 9.1 ± 4.0 $\mu g/cm^2$, respectively.

Effects on Stratum Corneum

In Vivo

Nook (1987) placed a water soluble ointment which contained 5% salicylic acid +10% Urea on the backs of six male subjects. The 4×4-cm squares (22/subject) were stained with 100 μl of a 1% silver nitrate solution followed by the application of 100 μl of Dektol. The treated areas were covered nonocclusively with Telfa and the ointment was left on the skin for 4 h. Reflectance of the skin areas was measured at 2, 24, 32, 48, and 56 h after application. The 5% salicylic acid + 10% Urea ointment had a higher bioavailability to the horny layer than the 5% salicylic acid ointment and the 10% salicylic acid ointment, but this difference was not statistically significant.

Horii et al. (1989) treated five patients with severe ichthyosis vulgaris and 10 patients with senile xerosis with a topical application of a 10% Urea containing cream daily for 2 to 4 weeks.

The extensor surfaces of the lower leg were used in the study. Surface samples of stratum corneum were obtained by a serial adhesive tape stripping. Samples of just adhesive tape were used as a control and subjected to the same analytical techniques as the treated samples. These control samples yielded a negligible amount of protein. Stratum corneum samples were also collected from the less dry areas of the flexor surface of the forearm of the elderly patients. The 10% Urea cream reduced chapping and scaling of the skin in all patients with ichthyosis vulgaris and senile xerosis. Clinical assessment produced a significant improvement in grade of patients with senile xerosis and ichthyosis vulgaris. No significant change was found in the amino acid content of the stratum corneum when treated with 10% Urea.

Sindhvananda et al. (1993) exposed the forearms of 109 volunteers to a 10% Urea cream base for five minutes. Hygroscopicity and water holding capacity were then measured. Water was applied for 10 sec, removed and the skin was measured again for hygroscopicity and water holding capacity. The water-holding capacity of the skin after exposure to all agents decreased in a slower manner than the control normal skin. After 5 min the hygroscopicity and water-holding capacity of the skin treated with Urea was almost twice that of the control. After 3 h the hygroscopicity and water-holding capacity of the skin treated with Urea was still above the control values, but was less than at 5 min.

Bettinger et al. (1994) exposed the forearms of 25 adults to water-in-oil (w/o) and oil-in-water (o/w) emulsion with and without 10% Urea. The test area (3/subject) had a diameter of 3 cm and was washed in the morning and evening during the study. Each area was treated three times a day for three days with each emulsion $\pm$ Urea. Both emulsions without Urea created a significant increase in corneal humidity compared to untreated skin; however, the w/o emulsion was more effective in preventing dehydration from the skin washing procedure. Both emulsions plus 10% Urea caused an increase in the horny layer hydration, but this difference was not considered significant.

Bettinger and Maibach (1997) exposed the heels of 54 adult volunteers to one of four formulations: group 1, 15 volunteers were exposed to w/o emulsion; group 2, 18 subjects were exposed to 10% Urea in the w/o emulsion of group 1; group 3, 10 subjects were exposed to o/w emulsion, and group 4, 11 volunteers were exposed to 10% Urea in an o/w emulsion. One heel was treated and the other served as the control in groups 1 and 3, whereas in groups 2 and 4 both heels were treated with control or Urea emulsions. The heel was treated with 2.5 g/cm^2 of the emulsion three times daily for 3 days. The soles were washed twice during each day of treatment. Three hours after the last application pieces of callus were mechanically removed from the sole. The calluses were exposed to RH (relative humidity) that started at 90% RH for 24 h and subsequently exposed to 60%, 70%, and 80% humidity for at least 1 day.

The callus pretreated with Urea had significant increases in water content at 90% RH compared to the callus treated with the control w/o or o/w emulsion. No significant differences with Urea treatment below 80% RH or less for either emulsion were observed. At RH of 60% and 70%, the water content of the stratum corneum was lower on the emulsion-treated side than on the non-treated side, regardless of the type of emulsion applied. The mean water content of the samples 6 h after collection was $33\% \pm 6\%$ (Bettinger and Maibach, 1997).

Clarys et al. (1999) applied test formulations to healthy skin. Two groups of 15 healthy male and female volunteers had halcinonide (a corticosteroid) at 0.005%, 0.050%, and 0.200% in an o/w vehicle and 5% Urea plus halcinonide 0.005% applied to a 3×3-cm area on the volar part of the forearm. The test formulations (3 mg/cm^2) were occluded with a plastic film and sealed with tape. Each formulation was compared to a contralateral blank treated with vehicle only. Formulations were applied in the evening and remained on the skin for 12 h. The first group of volunteers skin areas were reoccluded with plastic film during 2 h and at 34 and 82 h after initial application. The second group had a similar reocclusion procedure carried out at 58 and 106 h after initial application. Measurements were performed every hour for 5 h after removal of the reocclusions. All formulations were tested in the first group and only the 5% Urea plus halcinonide 0.005% and halcinonide 0.200% were tested in the second group.

In the first experiment, the 5% Urea plus 0.005% halcinonide and 0.200% halcinonide had significant blanching after the first reocclusion (34 h), but 0.005% and 0.050% halcinonide did not cause a blanching effect. After the second reocclusion (82 h), only 0.200% halcinonide had a significant blanching effect. In the second experiment, significant blanching occurred with the 0.200% halcinonide after the first (58 h) and second (106 h) reocclusion. No significant blanching was observed with the halcinonide plus Urea formulation at either of these times (Clarys et al. 1999).

In Vitro

Hellgren and Larsson (1974) performed In vitro experiments on the upper layer of human epidermis in order to evaluate water binding capacity. The tissue was dried to a constant weight and kept in 10% Urea, 5% sodium chloride, and distilled water solution for 1 h. The skin was transferred to a chamber with a constant humidity of 100% and a temperature of 25°C. The skin pieces were removed for weighing at designated time intervals. Keratolytic effects were also studied by placing the upper epidermis into saturated aqueous solutions of Urea and sodium chloride.

The water content, represented by weight increase, of the upper epidermis (1 to 2 mg) for distilled water, 10% Urea solution, 5% sodium chloride, and 10% sodium chloride was about 2.3, 5, 6, and 7 mg. The differences were statistically significant. Upper epidermis (5 to 10 mg) was also extracted with methanol:chloroform, 2:1. Distilled water, 10% Urea, 5%

sodium chloride, and 10% sodium chloride had weight increases of about 0.5, 3, 3.5, and 4.5 mg after extraction with methanol:chloroform.

The weight increase of unextracted skin in distilled water under the same conditions was about 1 mg. After 1 week, the Urea solution resulted in changes in the mechanical properties of the horny layer. After a few days, differences in the resistance towards unfolding due to streaming of the liquid was apparent. After about 1 month the quaternary structure of the keratin was lost. No effects were observed in the tissue pieces stored in sodium chloride after the same amount of time (Hellgren and Larsson 1974).

Van Duzee (1975) reported that overnight treatment of human stratum corneum samples with 0 to 5 M Urea demonstrated that the melting temperature for the protein component that denatured at 107°C was lowered monotonically as the concentration of Urea increased while the melting temperature for the α-keratin denatured at 85°C was unaffected.

Van Duzee (1978) studied the effect Urea on the elastic modulus of the stratum corneum (the quantitated ability of the stratum corneum to stretch in response to a force). The stratum corneum used in these experiments was obtained at autopsy from the midline section of female cadavers. A sample treated with 3 M Urea reduced the modulus at all water contents compared to the control. This same concentration of Urea produced stratum corneum which absorbed more water than the control at all relative humidities.

Thermal analysis studies used skin samples which were about 6 cm^2 in area. These samples were soaked overnight in 3 M Urea, then dried, rehydrated and sealed. Treatment with 3 M Urea produced a reduction of 15°C in the denaturation temperature of the nonfibrous intracellular protein component which suggested that Urea is binding to this component of the stratum corneum. The denaturation temperature of the α-keratin was not affected by the treatment nor were the two lipid components (Van Duzee, 1978).

Gournay et al. (1995) soaked stratum corneum samples from human thigh skin in 5% aqueous solutions of Urea for 24 h at room temperature. Five pieces of each sample were analyzed separately after the drying and hydration stages. Dried samples were evaluated after 24 h at 0% RH and rehydrated samples were evaluated after 72 h at 92% RH. The percent water content of the dried samples and rehydrated samples was $2.9\% \pm 0.6\%$ and $149.0\% \pm 0.7\%$, respectively.

Rao and Misra (1995) reported that the combination of a 10% Urea formulation plus pulsed DC (direct current) iontophoresis to human cadaver skin (HCS) produced a synergistic effect to improve the transdermal permeation of insulin across the HCS. In an earlier report, Rao and Misra (1994) stated that 10% Urea, 10% Urea +0.01% nonionic Pluronic F68, 10% Urea +0.1% sodium taurocholate, and 10% Urea +0.1% sodium tauroglycocholate significantly enhanced the permeation of insulin across HCS when pulsed DC iontophoresis was applied to the HCS.

Other Skin Effects

Lodén (1996) tested 72 healthy individuals with a 10% Urea emulsion (A), and with a 5% glycerin gel with 5 (B), and 10% (C) Urea below pH 7. Emulsion A also contained betaine, lactic acid, cholesterol, glyceryl monostearate, diethanolamine cetylphosphate, lanolin, sodium chloride, and water. Gels B and C also contained methylhydroxypropyl cellulose and water. The volunteers applied about 2 mg/cm^2 of the emulsion to 100 cm^2 of the volar aspect of the forearm. The study had two treatment periods. The longer period had a duration of 20 days, two applications daily and used an untreated skin area as the control, whereas the shorter period had three applications, the first was in the evening followed by one in the morning and one in the evening the next day, and used the vehicle as the control. Emulsion A and gel C were used for 20 days and gel C was also used in the shorter study. Gel B was applied three times in a double blind study.

TEWL and skin capacitance on the volar forearm were measured before entry into the 20-day study and on days 10 and 21. The measurements were made after completion of the treatments in the short-term study. The TEWL and skin capacitance were determined 10 to 20 h after the last application.

Treatment of the skin for 10 days with emulsion A and gel C increased the capacitance significantly compared with untreated controls. However, after 20 days the capacitance decreased for emulsion A and gel C. The TEWL was significantly decreased after 10 and 20 days for this emulsion and gel. Three applications of gel C caused a significant increase in skin capacitance, while three applications of gel B significantly increased TEWL. Three applications of emulsion A and gel C caused a significant reduction in TEWL, skin blood flow and visual signs of irritation caused by sodium lauryl sulfate. Skin capacitance and TEWL varied on the untreated control sites during the test period (Lodén 1996).

Clinical Treatment

Nash (1971) applied Urea (20%) to the feet of 75 patients with various dry skin pathologies. The patients were instructed to apply about 2 g of the cream to each foot once or twice daily and were observed for 7 months. Only one patient complained of stinging when the cream was applied to fissured areas.

Danopoulos and Danopoulou (1974a) treated patients with hepatomas with 2 to 2.5 g Urea orally 4 to 6 times daily. In the first case, a man with an egg-sized tumor, smaller nodules in both liver lobes and a palpable liver was treated with Urea for 10 months. The liver was not palpable 10 months after treatment with Urea and he lived at least 4 years and 9 months after the start of Urea treatment. In the second case, a woman with an egg-sized tumor that involved the liver and stomach was treated with Urea. After 4 months the nodules on the liver observed during laparotomy were impalpable and the egg-sized mass was half the size. The patient died 8 months later.

Danopoulos and Danopoulou (1974b) treated two patients with a 10% Urea solution every 2nd and 4th day with SC and

intracutaneous injections around squamous-cell and basal-cell carcinomas. In the first case, after 6 months of Urea injections around the tumor it disappeared completely. Six months later the biopsy was negative. In the second case that involved the basal-cell carcinoma, the biopsy after 18 Urea injections and daily application of Urea powder was negative. In 112 patients with squamous and basal cell carcinomas and malignant melanomas, 92% experienced great improvement or were completely cured with Urea treatment.

Danopoulos et al. (1975) treated eight patients with epibulbar malignancies with a 10% Urea solution. Most of the patients were completely cured and continued treatment with the Urea solution for at least 10 months.

Fredriksson and Gip (1975) treated subjects with ichthyosis and bilateral eczematous dermatitis (30 in each group) with two commercially available preparations that contained 10% Urea. Preparation 1 had a pH of 6 and preparation 2 had a pH of 3. The subjects applied the preparations to the upper and lower extremities for 4 weeks. A burning sensation was experienced upon application of preparation 2 in 13 patients but no side effects were noted with preparation 1.

Danopoulos and Danopoulou (1979) treated a 92-year-old man with a squamous cell carcinoma on the eyelid, cheek, inner canthus, and the caruncle with 29 injections of a 10% Urea solution. The authors reported that he was cured without any sequelae. The results were confirmed by three biopsies. A 75-year-old woman with a basosquamous cell carcinoma of the inferior eyelid was cured after receiving 33 total injections of a 10% Urea solution. A 75-year-old man with a large spindle-cell squamous carcinoma around the eye received four to six injections around the tumor every 2 to 3 days for 18 sessions. Further treatment with Urea powder every 2 to 3 days for 25 sessions over 4 months and continued injection of Urea cured the patient. The results were confirmed by three biopsies.

These same investigators reported using sc and intracutaneous injections of a 10% Urea solution to cure 46 patients with basal cell carcinoma, basosquamous cell carcinoma, and squamous cell carcinoma. Injections were repeated every 2 to 4 days for an average of 15 sessions, Urea powder was applied occlusively and patients were instructed to apply a saturated Urea solution to the treated area twice daily for at least 6 months.

Danopoulos et al. (1979) reported similar results as Danopoulos and Danopoulou (1979) using almost the same protocol to treat 9 patients with a 10% Urea solution. Eight of the nine patients were cured and most of the patients instilled Urea into the eye for 1 month to 1 year. Two patients had recurrent incidences, one of them had not followed advice to instill the Urea solution for 1 year after removal of the tumor.

Cramers and Thormann (1981) tested 79 patients, 28 male and 51 female, with eczematous skin disease with a cream that contained 10% Urea. The cream was tested under occlusion as a 48-h patch test. Positive patch test reactions were observed in 7/79 patients (9.9%) and another 5 had erythematous reactions on the 5th day after treatment. Five of the seven patients with positive patch test reactions had used the cream and only one had acute eczema. The other patients had a slight burning sensation when the cream was used on excoriated skin. Three of the patients with a positive patch test to the 10% Urea cream had other positive patch tests. A biopsy performed on one patient 72 h after application had slight basal spongiosis in the epidermis and moderate inflammatory infiltrate composed mainly of mononuclear cells around the capillaries in the superficial part of the corium. In the group with negative patch test reactions, 30 patients had used the 10% Urea cream previously and 28 had other positive patch test reactions.

Danopoulos and Danopoulou (1981) treated patients with primary hepatic carcinoma and metastatic liver malignancies with 12 to 15 g of pure Urea daily divided into six doses continuously. In two cases, Urea doses up to 30 g daily for periods of 9 to 29 months were given. Subjective improvement started within about 2 weeks of treatment with an increase in body weight, improvement of performance status, and sense of well-being. Regression of liver enlargements started in about 3 months. Patients (18) with primary liver carcinomas had median survival times of about 22 months. Patients with secondary liver carcinomas had median survival times of about 18 months.

Guerrier and Porter (1983) treated 34 children with mild to moderate psoriasis with a 0.1% dithranol plus 17% Urea cream twice daily for about 6 weeks. Assessments were made every 2 weeks during this time. The patients used a 2% salicylic acid ointment for about 4 weeks prior to treatment with the 0.1% dithranol plus 17% Urea cream. Side effects included inflammation of the normal skin surrounding the psoriatic lesion, stinging and/or burning of the skin, pruritus, and discoloration of the skin. Three patients withdrew from the study during the first week due to soreness or burning of the skin due to dithranol.

Decaux et al. (1985) treated a 45-year-old woman with alcoholic cirrhosis in icteroascitic decompensation with a daily Urea intake of 30 g over 6 days. She lost 3 kg and had an increase in serum sodium concentration and urinary sodium output without a change in creatinine clearance. Stopping the Urea treatment halted the weight loss but hyponatremia recurred. Another Urea treatment of 60 g/day for 2 days induced a weight loss of 4 kg in 3 days, induced the disappearance of clinical signs of salt retention, and increased serum sodium concentration to slightly above that of the previous treatment without impairment of glomerular filtration. Urine volume increased from 0.6 to 0.8 L/day to 1.0 to 1.5 L/day during treatment.

Gip and Lundberg (1985) treated 30 patients diagnosed with psoriasis twice daily for at least 3 weeks with a cream base (o/w emulsion) containing 12% NaCl or 12% Urea. The different cream bases were applied to different lesions on the same patient. Lesions were evaluated before the study initiation and at 1 and 3 weeks. No severe side effects were reported, although some patients complained of a burning sensation with both.

A study performed by Fredriksson and Lundberg (1985) compared the antipsoriatic effect of a cream containing 12% Urea and 12% sodium chloride with the cream vehicle in

40 psoriatic patients. The patients were specifically asked about adverse reactions, but no complaints were voiced.

Beverley and Wheeler (1986) treated 2 infants with a 10% Urea cream four times daily for collodion membrane. During the treatment periods the blood Urea concentration was elevated. The first infant was treated with the cream up to about day 62 and days 66 to 68. The resulting plasma Urea concentrations peaked at ~15 and 7 mmol/L, respectively. Plasma Urea decreased to normal concentrations upon cessation of use of the cream. The second infant was treated with the 10% Urea cream on days 0 to 4 and plasma Urea concentrations peaked at ~35 mmol/L.

Reproductive Effects

Weinberg and Shepard (1973) injected a 30% solution of Urea intra-amniotically to induce midtrimester abortion in 120 patients. After the removal of as much amniotic fluid as possible, 200 ml of the 30% Urea solution was infused by a gravity drip. Subjects then received 240 U of oxytocin at a rate of 100 ml/h.

Ninety-three percent of the patients aborted the fetus within 96 h after injection (the mean was 43.2 h). Blood Urea nitrogen (BUN) was measured in 10 patients every 2 h for 12 h after injection. Only one patient had a significant change in BUN immediately after injection. In the remaining nine patients, the BUN peaked at 4 h, was elevated until 8 to 10 h and dropped below 31 mg/100 ml by 12 h after injection. The BUN was evaluated every 4 h for 24 h in another 10 patients the same pattern was observed. Values for BUN obtained the morning after injection in an additional 35 patients ranged from 7 to 20 mg/100 ml. Preliminary results indicated no laboratory evidence of a coagulation problem (Weinberg and Shepard 1973).

Roopnarinesingh (1975) performed an intra-amniotic injection of Urea on eight patients with severe hypertension, 9 with severe pre-eclampsia and 3 with eclampsia during the second trimester. A 9-cm 16-gauge lumbar puncture needle was introduced through the abdominal wall into the amniotic cavity. After aspirating 50 to 100 ml amniotic fluid, 200 ml of a 30% solution of Urea was infused intra-amniotically at about 20 drops per minute. An intravenous drip of 100 units syntocinon in 1 L of 5% dextrose-water was started after the infusion with Urea was complete.

The fetus was expelled in 19 of the patients within 4.25 to 57.0 h after Urea infusion, with a mean of 24.25 h. The procedure was abandoned in one woman who had a severe backache, facial flushing and occipital headache at the onset of infusion. Three patients had a low-grade pyrexia and two patients had a generalized flushed sensation. No vomiting, rise in diastolic blood pressure, convulsions, or deaths occurred after Urea exposure.

Blood Urea estimations were performed in five patients at 4-hourly intervals during the induction of abortion. The initial blood Urea was 50 mg/100 ml and maximum Urea levels at 8 to 12 h ranged from 72 to 201 mg/100 ml (Roopnarinesingh, 1975).

Haning and Peckham (1985) dosed 10 female patients with Urea and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a study of the abortifacient properties of these chemicals. As much amniotic fluid as possible was removed prior to administration of drugs. Patients <20 weeks from their last menstrual period received 80 g Urea dissolved in 135 ml of 5% dextrose in water and 5 mg $PGF_{2\alpha}$ IA. Patients with pregnancies ≥ 20 weeks and up to 24 weeks from their last menstrual period were given 120 g Urea in 200 ml of 5% dextrose in water and 5 mg $PGF_{2\alpha}$ IA. The fetal heart was checked 2 to 3 h after instillation and patients with continued fetal heart activity after 3 h were given a second IA instillation of Urea and $PGF_{2\alpha}$. Venous blood was obtained prior to administration of the Ia drug and at 4, 8, 12, 24 h and after placental delivery.

The drug-fetus interval for 80 and 120 g Urea was 18.4 ± 5.9 and 14.8 ± 6.3 h, respectively. The fetus-placental interval was 1.01 ± 0.84 and 0.61 ± 0.84 h, respectively. The fetal heart rate was present in one fetus in the 80 g Urea group and in 6 fetuses in the 120 g Urea group at 2 h. Significant decreases in platelet count after placental delivery compared to baseline occurred in both groups. Fibrinogen also had a statistically significant decrease in the 80 g Urea group. No statistically significant differences between baseline and postplacental samples for prothrombin or partial thromboplastin time were demonstrated within the 80 and 120 g Urea groups.

Significant elevations in BUN occurred in both groups, peaked at 4 h and had returned to basal levels by 24 h. Serum sodium, potassium and carbon dioxide levels deceased slightly from baseline. No statistically significant alterations occurred in the serum chloride level (Haning and Peckham 1985).

Case Report

Lemière et al. (1996) reported that a 37-year-old flight attendant, with no history of respiratory complaints (although she had quit smoking 2 years prior to this incident), was exposed to Urea fumes that had been spread on a runway to prevent skidding of the aircraft during below-freezing temperatures. The fumes were generated during takeoff from the wheels on the runway and resulted in the combustion of the Urea and subsequent release of fumes. The flight attendant did not take any precautions to prevent exposure of herself to the fumes. She experienced a burning sensation in her throat, nose and chest at the time of inhalation of the fumes. One to 2 h later she started to experience a dry cough. The following days she experienced difficulty breathing accompanied by a burning sensation in the chest with wheezing. Six days after the accident she still experienced burning and difficulty breathing, although she was no longer coughing or wheezing. She did complain of nasal congestion.

A bronchoscopy was performed 18 days after the accident. The bronchial mucosa had discrete congestion. Bronchoalveolar lixiviation was normal. Bronchial biopsies revealed an epithelial coating with some foci of hyperplasia of the mucus cells and some atrophic areas with no ciliated cells. The basal membrane was slightly thickened. The subjacent conjunctive tissue had

inflammatory infiltrate with lymphocytes and a few polynuclear neutrophils and eosinophils. Moderate fibrosis of the chorion was also observed. Immunodetection with L26 and EG2 was negative, whereas CD3 reported numerous lymphocytes in the chorion and epithelial coating. Respiratory function 2 months after the accident, which included studies of pulmonary volume, spirometry, diffusion of arterial gases, and bronchial reactivity to methacholine, revealed slight bronchial hyperexcitability as the only anomaly. Four months after treatment with inhaled steroids the patient was asymptomatic and bronchial activity was normal (Lemière et al. 1996).

SUMMARY

According to the *International Cosmetic Ingredient Dictionary and Handbook*, Urea functions in cosmetic products as a buffering agent, humectant, and skin-conditioning agent—humectant. Other sources add desquamating action and antimicrobial action to that list. FDA reported that Urea was used in 239 formulations in 2001. Concentrations of use reported by industry ranged from 0.01% to 10%.

Urea is considered GRAS by FDA and is accepted for the following uses: side-seam cements; as an inhibitor or stabilizer in pesticide formulations and formulations applied to animals; as internal sizing for paper and paperboard, and as 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 [^{15}N]Urea and [^{14}C]Urea (12 to 20 mg together) injected into rabbits was 3.05 and 2.50 h, respectively. The reutilization rate of Urea (differences in synthesis rate/Urea catabolism rate measured with ^{14}C) were 32.2% to 88.8%. The Urea excretion rates ranged from 30.4 to 26.6 mg Ci/h in one study and from 8.2 to 38.4 mg/h, with delay time variations of 0.33 to 1.05 h, in another.

[^{14}C]Urea was rapidly absorbed into monkeys after intraperitoneal injection; the serum concentration peaked within 30 min. The average intravenous concentration of Urea was 375 mg/dl 4 min after the end of the 3.5-min injection. As venous concentrations of Urea decreased exponentially, the half-life was 5 to 6 h. A similar rate of elimination was observed for intraperitoneal and intra-amniotic injections. The apparent volume of distribution was 70% to 85% of body weight.

The following regions of the brain of Osbourne-Mendel rats had approximately equal concentrations of radioactivity after a bolus injection or continuous infusion of [^{14}C]Urea for up to 40 min: frontal lobe, caudate nucleus, hippocampus, thalamus plus hypothalamus, pons, and white matter (corpus callosum).

The Urea pool of humans varied from 13 to 20 g; the rate of production from 0.96 to 1.96 g/h; and metabolism varied from 0.15 to 0.43 g/h. Serum Urea concentrations in subjects given 30 g of Urea rose from 36.4 ± 4.74 mg% to a maximum of 97.6 ± 9.17 mg% after 40 min, and remained high at 94.6 ± 8.72 mg% after 1 h. The mean Urea half-life was $8.3\% \pm 0.9\%$ h.

The permeability constant after treatment with Urea of whole skin and the dermis of rabbits was 2.37 ± 0.13 ($\times 10^6$) and 1.20 ± 0.09 ($\times 10^3$) cm/min, respectively. The absorption of Urea across normal and abraded human skin was $9.5\% \pm 2.3\%$ and $67.9\% \pm 5.6\%$, respectively.

Urea increased the skin penetration of other compounds.

No female rats or mice died and toxicity was not observed when acutely dosed with 200 mg/kg Urea and 1000 or 2000 mg/kg Urea, respectively. 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 that lead to a deep torpor or coma. Male, cross-Landrance piglets were dosed orally with 1, 2, and 4 g/kg Urea for 5 days. All piglets remained healthy throughout the study and no signs of toxicity were observed. Male nude mice were dermally exposed to 1%, 10%, 25%, 50%, 75%, or 100% Urea for 24 h. No significant microscopic changes were observed.

A statistically significant improvement occurred in eyes with toxic keratitis after Urea (2.24%) was applied to the conjunctival sac of the eye for 11 days.

Urea injected directly into the uterine horn of female Sprague-Dawley rats was an effective contragestational agent on day 3 (preimplantation) of gestation, but not day 7 (postimplantation). None of the concentrations of Urea produced any maternal mortality or morbicity. Male BALB/c mice received five daily intraperitoneal injections of Urea (250, 500, 1000, or 2000 mg/kg/day). Urea did not significantly increase the incidence of abnormal sperm heads. 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. Urea (50 mg/kg) given orally with 10 mg/kg *N*-nitrosomethylurea (NMU) to rats on the 9th day of pregnancy did not effect embryonic mortality, frequency or character of the deformities induced by NMU. ICR-JCL mice dosed with a detergent containing 15% Urea dermally had no toxic effects. There were no significant differences between the control and Urea-treated groups with respect to implantations, live fetuses, sex ratio, and body weight of live fetuses. The same detergent (15% Urea) was used and injected into pregnant ICR-JCl mice and dams and fetuses had no significant differences when compared to control animals.

Urea was not genotoxic in several bacterial and mammalian assays. 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, but this property has not been linked to any

genotoxic activity. Urea was not carcinogenic in Fisher 344 rats or C57B1/6 mice fed diets containing 0.45%, 0.9%, and 4.5% Urea.

Although one report found 60% Urea applied to normal skin to be nonirritating, 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. A 10% Urea cream was patch tested in 79 patients with eczematous skin disease for 48 h. Positive patch test reactions were observed in 7/79 patients (9.9%). Overall, there are few reports of sensitization among the many clinical studies that report use of Urea in treatment of diseased skin.

Urea injected intra-amniotically induce mid-trimester abortions in humans.

In several patients treated with 10% Urea solution subcutaneous and intracutaneous injections around squamous-cell and basal-cell carcinoma experience great improvement or complete cure with Urea treatment.

DISCUSSION

The CIR Expert Panel determined the data provided in this report to be sufficient to assess the safety of Urea. Acute, short-term, and chronic animal toxicity studies demonstrate little evidence of adverse effects, even at high exposures. Dermal irritation, for example in the nude mouse, was not significant. Urea treatment of corneal damage resulted in an improvement in one animal study. Reproductive and developmental toxicity studies in animals raised no concerns.

The Panel did note that formulators should be aware that Urea can increase the percutaneous absorption of other chemicals. The Panel also discussed that Urea can cause uncoiling of DNA, a property used in many DNA studies. This property may explain many of the genotoxicity assays in which Urea was found to be genotoxic at high concentrations. Overall, Urea is not expected to have any in vivo genotoxic activity. Urea was not carcinogenic in the limited studies that are available.

This ingredient is reportedly used in some product categories, but the concentrations of use are not available. In other cases, information regarding use concentration for a specific product categories is provided, but the number of such products is not known.

Although there are gaps in knowledge about product use, the overall information available on the types of products in which this ingredient is used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers Urea to be safe.

CONCLUSION

Based on the available data contained in this report, the CIR Expert Panel concluded that Urea is safe as used in cosmetic products.

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