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Acute Toxicity of Uranyl Nitrate to Growing Chicks: A Pathophysiologic Study

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Uranium salts, administered parenterally, were first recognized by Leconte to be nephrotoxic in 1854 (McNider 1929). Since that time, uranyl nitrate (UN) has been the principle uranium compound used for mammalian nephrotoxic modeling (Avasthi et al. 1980; Dickson 1909; Flamenbaum et al. 1974; Haley 1982; MacNider 1929; Pelayo et al. 1981; Stein et al. 1975). Uranium is present *in vivo* in tetravalent (U^{4+}) or hexavalent (U^{6+}) forms, with U^{6+} as the most stable valance. Tissue deposition of uranium occurs primarily in bone and kidney while the chief route of excretion is via the urine (Passow et al. 1961). Uranyl nitrate causes injury and necrosis of the renal tubular epithelium (Hodge et al. 1973; Tannenbaum 1951; Voegtlin and Hodge 1949).

A suitable nephrotoxic compound was required for validation of an impaired renal function (IRF) model developed by Harvey et al. (1984). While UN has been used extensively for nephrotoxic modeling, toxicity data have not been available delineating the effects of UN on chicken cells or tissues. The objective of this study was to characterize the pathophysiologic effects of UN in growing chicks.

MATERIALS AND METHODS

Four hundred one-day-old, Leghorn cockerals (Hy-Line, W-36) were individually wingbanded and housed in heated batteries (20 chicks/deck). The chicks received continuous light and were fed commercial starter ration and tap water *ad libitum*. When four weeks old, 320 chicks were randomly selected and assigned to treatment groups of 20 chicks/group of untreated controls, saline controls, 70, 100, 130, 160, 190, 220, 250, 280, 310, 340, 370, 400, 430, or 460 mg UN/kg of body weight. Uranyl nitrate, 6-hydrate (Fisher Scientific Products, Houston, TX) was dissolved in physiological saline and concentrations (wt/v) calculated on UN content less the hydrated portion of the compound. Dosages were administered subcutaneously at the base of the neck via a 22 ga needle and tuberculin syringe. Concentrations of UN solutions were adjusted so that volumes of injections did not exceed 1.2 ml/chick. Saline controls were injected subcutaneously with

1.2 ml physiological saline. Group mortality was monitored for 7 days and an LD_{50} calculated (Reed and Muench 1938). All chicks that died were necropsied and examined for gross lesions.

A dose versus time response was initiated to determine the sequential effects of 250 mg UN/kg body weight from 12-96 hrs post-injection. Three hundred, one-day-old Leghorn cockerals (Hy-Line, W-36) were individually wingbanded and placed in heated batteries (20 chicks/deck). The chicks received continuous light and were fed commercial starter ration and tap water ad libitum. When 3 weeks old, 250 chicks were randomly selected and assigned to 2 treatments of 125 chicks/group. Treatments consisted of untreated controls (Group 1) and those injected subcutaneously with 250 mg UN/kg body weight (Group 2). At 0, 12, 24, 48, and 72 hrs post-injection, 15 chicks from each treatment group were bled for biochemical analyses via cardiac puncture, killed by cervical dislocation, and subjected to necropsy. Liver and kidney tissues were collected from 5 chicks per treatment at 12, 24, 48, 72, and 96 hrs post-injection, placed in 10% neutral buffered formalin, paraffin embedded, sectioned at 5 microns, stained with hematoxylin and eosin, and examined for microscopic lesions. For electron microscopy, tissue samples of kidney were minced in fixative containing 2% glutaraldehyde, 0.05 M PIPES buffer (pH 7.3), and 0.05 M sucrose and post fixed in 1% osmium tetroxide, PIPES buffer, and sucrose. The tissues were rinsed, dehydrated and embedded in epoxy resin. Ultrastructural examination was performed with a Phillips EM 300 microscope. Serum biochemical measurements for Ca, Mg, K, Na, uric acid (UA), creatinine (Creat), albumin (Alb), BUN, cholinesterases (ChE), gamma glutamyl transferase (GGT), inorganic phosphorus (P), total protein (Tot Prot), alkaline phosphatase (Alk Phos), lactic dehydrogenase (LDH), aspartate aminotransferase (AST, SGOT), and creatine kinase (CK) were performed on automated equipment according to manufacturer's recommendations and have been described elsewhere (Harvey et al. 1984). All data for biochemical analyses were compared statistically according to the general linear model procedure for analysis of variance and were ranked according to the Duncan's multiple range test (SAS Institute, Inc., 1982).

RESULTS AND DISCUSSION

The LD_{50} for UN, based on 7 day mortality, was 235 mg/kg body weight, while the lowest dosage that caused death was 160 mg/kg of body weight. These dosages approximate 100-200 times the 14-21 day LD_{50} for rats (1-2 mg/kg body weight IP) and approach the 24 hr LD_{50} of 135-305 mg/kg body weight for rats (Voegtlin and Hodge 1949). These data suggest that growing chicks are relatively insensitive to the lethal nephrotoxic effects of UN. Chick mortality was highest on days 3-5 post-injection. Upon necropsy, the outstanding post-mortem lesions included visceral gout; thickened peritoneal and air sac membranes; swollen mottled

kidneys; and a dark swollen, congested liver. A characteristic putrid odor was noted in uricemic chicks upon opening the abdominal cavity. Tissues at UN injection sites were edematous with yellow staining of the subcutaneous fat, connective tissue and musculature.

Mortality in the dose vs. time study did not begin to appreciably increase until after 48 hrs. Gross lesions at necropsy were similar to those noted in the LD_{50} study. Serum biochemical measurements (Tables 1, 2, and 3) demonstrated that differences between groups existed as early as 12 hrs post-injection. Observed changes of enzymes (Table 1) included decreased GGT at 48 hrs, decreased Alk Phos 12-48 hrs, decreased ChE 12-72 hrs, increased CK 12-72 hrs, increased AST 48-72 hrs (Fig 1), and increased LDH 48-72 hrs (Fig 1). Serum metabolite values (Table 2) demonstrate increased UA and BUN 12-72 hrs; increased CREAT 12, 48, and 72 hrs; decreased Tot Prot at 12, 48, and 72 hrs;

Table 1. Serum enzymatic activities of chickens 12-72 hrs post-injection with 250 mg UN/kg body weight[1]

Time Post-Dosing (hrs)	Treatment[2]	Parameter			
		GGT	Alk Phos	ChE	CK
		IU/l			
0	Gp 1	14.8^{bc}	1460^{abc}	845^{a}	1186^{de}
12	Gp 1	11.9^{de}	2059^{a}	878^{a}	1264^{de}
	Gp 2	10.4^{e}	872^{cd}	571^{b}	4163^{a}
24	Gp 1	16.4^{abc}	1560^{ab}	812^{a}	1317^{de}
	Gp 2	14.1^{cd}	778^{d}	579^{b}	3697^{a}
48	Gp 1	16.4^{abc}	1460^{abc}	859^{a}	1581^{d}
	Gp 2	12.0^{de}	771^{d}	491^{b}	2975^{b}
72	Gp 1	18.3^{a}	1559^{ab}	868^{a}	974^{e}
	Gp 2	17.0^{ab}	1043^{bcd}	522^{b}	2140^{c}

1 Means of 15 chicks per treatment
2 Gp 1 = untreated controls; Gp 2 = UN injected
3 GGT = gamma glutamyl transferase; Alk Phos = alkaline phosphatase; ChE = cholinesterases; CK = creatine kinase;
a,b,c,d,e In the same vertical column, means followed by different superscripts differ significantly ($P<0.05$).

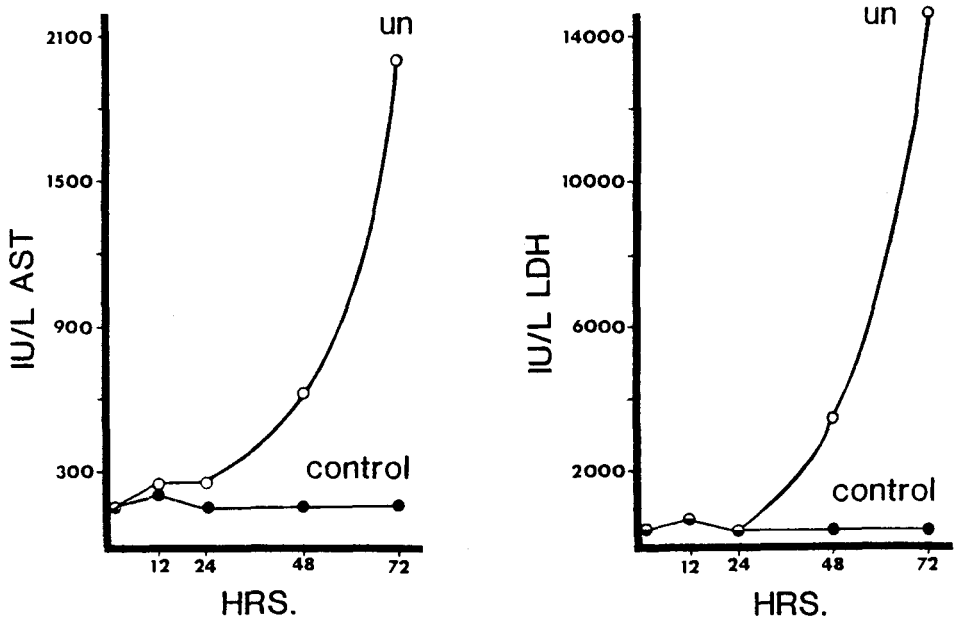

Figure 1. Serum enzymatic activities of AST(SGOT) and LDH of chicks injected with 250 mg UN/kg BW

and decreased Tot Prot and Alb at 12-72 hrs. Of the serum minerals evaluated, Ca decreased at 12 and 24 hrs; K decreased at 72 hrs; Mg was decreased at 12 and 24 hrs, unaltered at 48 hrs, and increased at 72 hrs; Na was decreased at 24-48 hrs; and P was increased at 12 and 24 hrs.

Microscopic examination demonstrated mild focal proximal convulated tubular degeneration was present in kidneys of Group 2 by 12 hrs. By 24 hrs, focal tubular degeneration and necrosis, tubular degeneration with casts, and some regeneration were present. No significant hepatic lesions were observed. At 48 hrs, renal lesions included moderate to severe nephrosis, cellular and protein casts, and some regeneration. Liver lesions ranged from mild focal necrosis to severe multifocal necrosis. At 72 hrs, renal examination revealed mild to moderate nephrosis with regeneration more evident. Severe hepatic necrosis was diagnosed in liver sections. By 96 hrs, no major lesions, except occasional mitotic figures in kidney sections, were observed.

Ultrastructural examination of kidney tissues revealed changes in cellular and subcellular architecture, occurring most frequently at 48, 72, and 96 hrs post-dosing. Affected cells exhibited a pattern of general disorganization, minor changes in mitochondrial size and shape, disruption of endoplasmic reticulum, slight increases in the number and size of lipid droplets, and some loss

Table 2. Serum metabolite values of chickens 12-72 hrs post-injection with 250 mg UN/kg body weight

Time Post-Dosing (hrs)	Treatment[2]	Parameter				
		UA	BUN	Creat	Tot Prot	Alb
		mg/dl			g/dl	
0	Gp 1	5.16 e	.94 f	.48 c	2.77 ab	1.25 bc
12	Gp 1	7.02 e	2.00 cd	.53 bcd	2.74 ab	1.24 bc
	Gp 2	20.07 a	2.79 ab	.61 a	2.44 c	.94 e
24	Gp 1	5.26 e	1.49 def	.56 ab	2.87 a	1.14 d
	Gp 2	13.25 bc	2.30 bc	.59 ab	2.77 ab	.99 e
48	Gp 1	5.44 e	1.39 ef	.48 c	2.88 a	1.37 a
	Gp 2	11.11 cd	2.45 bc	.58 ab	1.95 d	.75 f
72	Gp 1	4.66 e	1.56 de	.54 bc	2.75 ab	1.30 ab
	Gp 2	16.39 ab	3.16 a	.61 a	1.88 d	.77 f

[1] Means of 15 chicks per treatment
[2] Gp 1 = untreated controls; Gp 2 = UN injected
[3] UA = uric acid; BUN = blood urea nitrogen; Creat = creatinine; Tot Prot = total protein; Alb = albumin
a,b,c,d,e,f In the same vertical column, means followed by different superscripts differ significantly ($P<0.05$).

of microvilli. There was, however, much diversity in tissue responses between treated chicks.

Serum enzymes, metabolite, and mineral data for all sampling times agree closely with a previous study when samples were drawn only at 48 hrs (Harvey et al. 1986). Cholinesterases are synthesized in the liver, and hepatocellular necrosis, noted above, should tend to cause a decrease in production of this enzyme. On the other hand, acute hepatic and renal necrosis would predictably cause an increase in LDH, AST, and CK, since these organs (and others) possess high intracellular concentrations of those enzymes (Hook 1981; Sturkie 1976; Kramer 1980). Decreased Alk Phos, although not readily explained, could be related to the replacement of Ca in bone by uranium (Passow et al. 1961). The major source of Alk Phos in young growing animals is from osteoblasts, and perhaps uranium toxicity to osteoblasts disrupted Alk Phos production (Kramer 1980). Increased UA, BUN, CREAT, and P levels, all indicators of renal insult in birds and mammals (Harvey et al. 1984; Harvey et

Table 3. Serum mineral values of chickens 12-72 hrs post-injection with 250 mg UN/kg body weight

Time Post-Dosing (hrs)	Treatment [2]	Parameter				
		Ca	K	Mg	Na	P
		mg/dl				
0	Gp 1	11.90^{a}	19.43^{abc}	2.68^{bc}	348^{ab}	6.78^{d}
12	Gp 1	11.92^{a}	22.11^{a}	2.80^{ab}	362^{a}	7.27^{cd}
	Gp 2	10.46^{b}	20.76^{abc}	2.36^{def}	353^{ab}	9.86^{a}
24	Gp 1	11.79^{a}	20.11^{abc}	2.58^{cd}	363^{a}	6.28^{d}
	Gp 2	10.02^{b}	17.31^{c}	2.24^{f}	344^{bc}	10.16^{a}
48	Gp 1	11.87^{a}	21.38^{ab}	2.46^{cdef}	363^{a}	8.01^{bc}
	Gp 2	11.93^{a}	17.83^{bc}	2.42^{def}	329^{c}	8.66^{b}
72	Gp 1	11.23^{a}	22.15^{a}	2.32^{ef}	356^{ab}	6.88^{cd}
	Gp 2	11.53^{a}	13.41^{d}	2.91^{a}	346^{ab}	7.00^{cd}

1 Means of 15 chicks per treatment
2 Gp 1 = untreated controls; Gp 2 = UN injected
3 Ca = calcium; K = potassium; Mg = magnesium; Na = sodium; P = inorganic phosphorus
a,b,c,d,e,f In the same vertical column, means followed by different superscripts differ significantly ($P<0.05$).

al. 1986; Simeson 1980), suggests the acute nephrotoxic action of UN on tubular function.

Reduced Tot Prot, Alb, and K measurements throughout sampling would indicate tubular integrity was disrupted since these molecules are reabsorbed in the proximal convoluted tubules (PCT) (Hook 1981; Siller 1981; Sturkie 1976). In mammalian studies, UN is toxic to PCT cells and binds with Alb to impair tubular protein transport (Hook 1981). In the present study, Alb probably accounted for the greatest amount of Tot Prot losses, as it is routinely filtered by the glomerulus, then reabsorbed by the PCT. Loss of renal tubular integrity would tend to reduce the ability to reabsorb K and may account for the decrease in serum K levels. Additionally, high production and secretion rates of urates may have accelerated K ion loss, as K is required for UA tubular transport in chickens (Austic 1983). In contrast, increased serum K levles are observed following mammalian renal tubular injury (Hook 1981).

The PCT appears to be the primary site of UN toxicity in chickens as evidenced by serum biochemical measurements and histopathology. These results agree generally with mammalian studies discussed earlier. However, a major difference between mammals and chicks is the LD_{50} of UN. The 7 day LD_{50} of chicks is approximately one hundred-fold the 14 day LD_{50} of rats and mice.

Hepatotoxicity, a predominate feature of chickens, has not been described in mammalian species. The most dramatic indicators of hepatotoxicity in the present study are elevations in serum activities of AST(SGOT) and LDH (Fig 1). These enzymes are increased in a highly significant manner, suggesting that hepatic necrosis is extensive following UN dosage. Pathological lesions of the liver confirm clinical and serum biochemical suspicions of hepatotoxicity. Possibly the renal-portal shunt of avian species (Sturkie 1976; Sperber 1946), allows the liver to spare the kidney from toxicants at the expense of the former. Unfortunately, organ residue analyses were not performed, so it is unknown whether uranium is sequestered in the liver of chickens. In mammalian studies, 20-90% of uranium is deposited in bone and kidney, while only approximately 1% is found in the liver (Neuman et al. 1948; Hamilton 1948; Prister 1969). A recent study involving Japanese quail (Robinson et al. 1984), indicates that uranium deposition in the livers of quail is no greater than that found in mammals. Although it is possible that hepatic necrosis in chickens in the present study is simply a reflection of acute uric acid toxicity, no urate crystals are noted in necrotic hepatic foci as are described for visceral gout (Siller 1981). In the present study, regeneration of necrotic hepatocytes occurs within 96 hrs of UN dosing and suggests that the avian liver recovers from injury quite rapidly.

In spite of the chicken's relatively refractive response to administration of acutely toxic concentrations of UN, abnormal biochemical measurements and renal and hepatic lesions persisted for 72 hrs post-dosing. Light microscopic examination suggests that renal and hepatic architectures were returning to normal at 96 hrs post-dosing, whereas, electron microscopic observations demonstrate that ultrastructural changes still exist. These pathophysiologic data should prove helpful in providing basal data for nephrotoxicity studies in avian species.

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