ACUTE TOXICITY OF URANYL NITRATE AND BEHAVIOUR OF SERUM LIPOLYTIC ACTIVITY

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Summary : The alterations in the serum lipolytic activity of mice were studied under Uranyl nitrate (UN) intoxication. The lipemia produced as a result of UN intoxication was probed studying the alterations in the serum triacyl-glyerol-hydrolase (EC 3.1.1.3) activity. After an intraperitoneal injection of UN (10 *mg/kg* and 25 *mg/kg*), triacyl-glycerol-hydrolase (TAGH) activity was considerably enhanced. In the initiation phase the elevation in the activity was observed to be higher than the elevation in the maintenance phase of acute UN toxicity. The possible reasons for the elevation in the TAGH activity and its role under such intoxication is discussed.

Key words : uranyl nitrate acute toxicity lipemia TAGH activity

INTRODUCTION

During the past two decades, extensive studies have been made on Uranium poisoning, however, the manner by which UN exerts its effects on cellular metabolism remains obscure. Bauer *et al.* (2) have reported lipemia and azotaemia following the administration of UN in rabbit. Prior to that Tripodo (15) has reported a gradual progressive increase in the lipolytic power of plasma under UN toxication which reached it's maximum between 1st and 2nd week of the treatment.

In the recent years many reports on acute renal failure (ARF) caused by UN have been published (1,3,6,14,18). The above reports have thrown light upon the effect of UN on renal dysfunction (ARF) and mechanism of UN toxicity but no studies have been done to gain an insight into the lipid metabolism. We have earlier reported a presumptive role of lipase in the mechanism of UN toxicity in adipose tissue (8) in kidney and liver (5) where a remarkable elevation in the lipolytic activity was observed in response to the acute UN toxicity. The present study was undertaken to examine the relationship between adipose tissue, kidney, liver and blood under such condition and

obtain a more complete picture of sequential biochemical alterations taking place during acute UN toxicity.

MATERIAL AND METHODS

Chemicals – Triolein was obtained from Sigma Chemicals U.S.A., Diphenyl Carbazid was from E. Merck Dermsteadt Germany. Diphenyl Carbazone was from Veb. Janapharm Laborchieme, Alpoda, Germany. Tris (hydroxymethyl) aminomethane was from BDH Chemicals Pvt. Ltd., Poole, England. Other chemicals used in lipase assay were of analytical grade available commercially.

Animals and administration of UN : Male white mice (Kasauli strain) weighing 25-30 *gm* had free access to rat pellet feed and water which was administered *ad libitum*. Animals were further divided into 3 groups, each group consisting of 50 animals.

Experimental protocol

Male white mice
(Body wt. 25-30 *gm*)

GROUP A	GROUP B	GROUP C
Pellet feed, tap H_2O	Pellet feed, tap H_2O	Pellet feed, tap H_2O
(control)	(UN treatment)	(UN treatment)
Dose - 0.9% Saline	Dose - 10 *mg/kg* UN	Dose – 25 *mg/kg* UN

Uranyl nitrate ($UO_2 (NO_3)_2 . 6H_2O$) BDH Chemicals Pvt. Ltd., Poole (England) was dissolved in a concentration, such that each animal received 1 *ml* of intraperitioneal solution per *kg* body weight. Control group received a equivalent volume of 0.9 percent isotonic saline. After treatment, mice were returned to metabolic cages and sacrificed after desired time interval.

Biochemical studies : Animals were anaesthesized after 1,2,4,8,12,24,48 and 72 hr of injection and blood samples were collected directly by heart puncture in pre-oxalated tube and were diluted suitably with 1% saline (11). Centrifugation under 3000 x g was carried out for 15 min and the supernatent was taken out for further enzyme assay.

Enzyme assay : The lipase (TAGH) was assayed by the method discribed by Hayashi and Tappel (10). The assay system contained 0.25 *ml* of substrate (Triolein 0.4 *mM*), 1.5 *ml* of Tris-HCl buffer (pH 7.2) and 0.1 *ml* of suitably diluted enzyme sample with 0.4 *mM* Diisopropyl-flurophosphate. The incubation was carried out in a metabolic shaker with constant shaking at the rate of 160 strokes per minute with 4 *cm* amplitude for 10 min at 37°C. The enzyme reaction was terminated at the end of incubation by adding 2 *ml* of copper-TEA (Copper nitrate, Triethanolamine and acetic acid 10 : 9 : 1) reagent, 10 *ml* of chloroform was added to above content, shaked vigrously and allowed to settle down for 1½ hr. The liberated FFA were measured colorimetrically according to Itaya (11).

Protein estimation : Protein was determined according to Lowry's method (12).

RESULTS

Physical observations : Within 24 to 48 hr after UN injection (10 *mg/kg* and 25 *mg/kg*), animals became inactive, responseless and limpness was persistent throughout

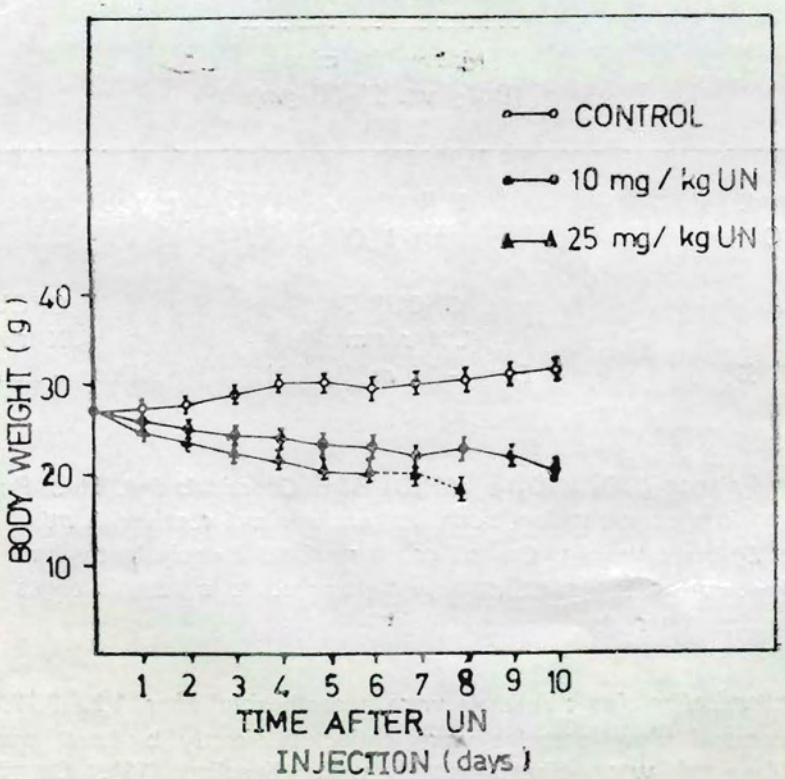

Fig. 1 : Rate of decrease in body weight of experimental models (low dose and high dose) after different timei ntervals.

O—O Control ●—● 10 *mg/kg* UN ▲-▲ 25 *mg/kg* UN

the later period. They showed very little interest in food and water resulting in loss of body weight (Fig. 1). Gradually they became moribund and died in coma. Mice treated with 10 *mg/kg* dose of UN, became moribund about 6 days after the treatment and mortality was maximum between 7-11 days. With high dose of UN (25 *mg/kg*) mortality was maximum between 5-8 days.

Biochemicals results : The biochemical results of serum lipolytic activity (TAGH) under UN intoxication gave remarkable results in both experimental models. Table I shows the elevation in lipolytic activity (TAGH) after a low dose of 10 *mg/kg* and a high dose of 25 *mg/kg* of UN after various time intervals.

TABLE I : Alterations in serum lipolytic activity under low dose (10 *mg/kg*) and high dose (25 *mg* per *kg* of Uranyl Nitrate).

Hour	Control TAGH activity		Dose 10 mg/kg. UN TAGH activity		Dose 25 mg/kg. UN TAGH activity	
	Units//ml	Units/mg protein	Units/ml	Units/mg protein	Units/ml	Units/mg protein
1	0.411±0.0041	1.284±0.0058	0.600±0.10***	2.615±0.010	0.585±0.0076***	2.564±0.020
2	0.420±0.0068	2.160±0.0053	0.600±0.12***	2.500±0.013	0.570±0.0086***	2.375±0.016
4	0.405±0.0042	2.100±0.0089	0.555±0.0057***	2.971±0.0093	0.540±0.0062***	2.250±0.019
8	0.390±0.0052	1.404±0.0077	0.510±0.0070***	2.710±0.020	0.480±0.0085***	2.285±0.028
12	0.420±0.0050	1.750±0.0070	0.525±0.0075***	3.144±0.013	0.495±0.0085***	2.770±0.027
24	0.420±0.0047	1.825±0.0082	0.516±0.0045***	3.710±0.015	0.480±0.0060***	2.905±0.0097
48	0.441±0.0066	2.68 ±0.012	0.516±0.0048**	3.900±0.035	0.456±0.0043*	2.980±0.0058
72	0.390±0.0035	2.290±0.011	0.465±0.0045***	3.144±0.012	0.405±0.0058**	2.738±0.012

All values are Mean ± S.E. of 5 mice.

Lipase (TAGH) activity is expressed as *μmoles;* Free fatty acids released per *ml* tissue per min.

P values *** $P<0.01$

** $P<0.05$

* $P<0.1$

A gross idea of level of serum lipolytic activity under low and high dose of UN at different time intervals is depicted in Fig. 2.

During the early initial phase (1, 2 hr) of UN toxicity, serum TAGH activity exhibited a marked elevation. The lipolytic activity was elevated to about 45% percent ($P<0.01$). This elevation was observed in both the experimental models. However, the elevation in

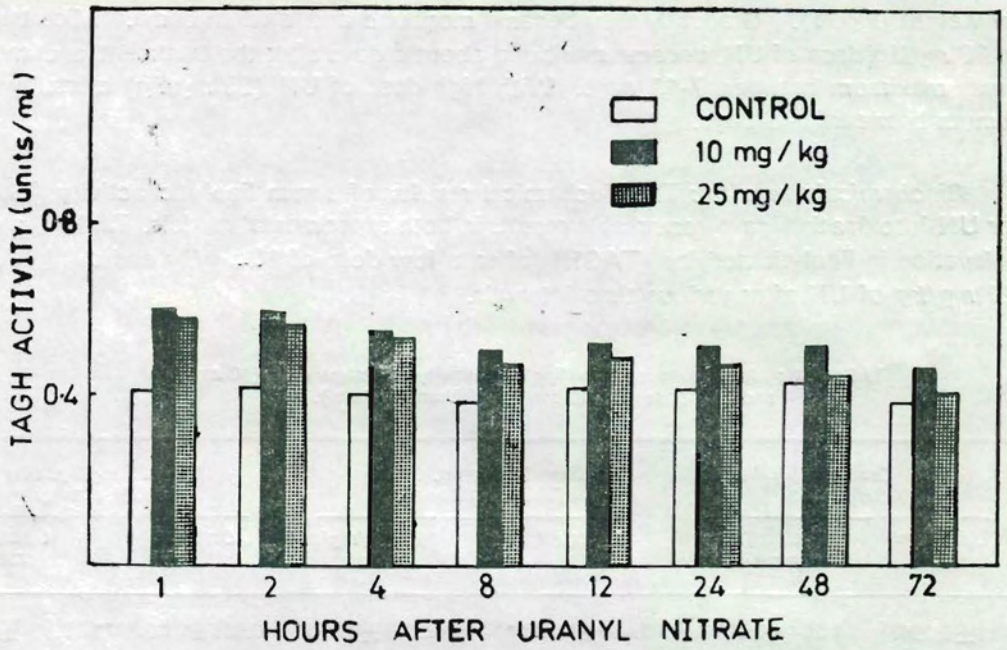

Fig 2 : Alterations in TAGH activity (*Units/ml*) following administtration of Uranyl nitrate in experimental models.

•lipolytic activity is expressed as units of free fatty acids released per *ml/min.*

•All the values are mean ± S.E. of 5 animals.

the TAGH activity of high dose model (25 *mg/kg*) was found to be less as compared to the low dose model (10 *mg/kg*).

In the next phase of UN toxicity (4,8,12 hr) the augmentation of lipolytic activity was persistent (25-30%, $P<0.01$) but there was fall in the elevation of TAGH activity (about 15%). In the maintenance phase this fall in TAGH activity gradually increased to about 25 percent.

DISCUSSION

The sublethal dose of 10 *mg/kg* UN and lethal dose of 25 *kg/mg* UN were sufficient to produce lipemia. These results were simultaneously manifested along with predetectable morphologic changes in kidney, liver and brain (5). Thus, the above doses provided an excellent experimental model for the study of lipolytic changes in mice serum.

The accumulation of triglyceride and cholesterol ester is delicately balanced by the liver in normal conditions, development, of "fatty liver" in pathogenic condition imbalances

the uptake of lipids from blood and synthesis of lipoproteins in the liver and consequently lipemia is developed. In this regard some suggestive evidence for above mentioned fact has been adduced recently by our laboratory in Rat. Where a significant rise in the cholosterol ester and triglyceride level of serum was observed within 24 hrs of the UN administration. The role of TAGH in such condition is both interesting and encouraging.

The elevation in TAGH activity after the administration of UN (10 *mg/kg* and 25 *mg/kg*) is most significant (45%, $P>0.01$) in the early initiation phase (1,2 hr.). It is probably due to the toxic shock encountered by the animal (7,9). The other possibility of localized effect of UN, which hydrolyzes to produce acid and cause the local effects of this compound (4) may give the probable explaination to the toxic shock encountered by the animal under UN intoxication.

During the late initiation phase (4,8, 12 hr) the augmentation in lipolytic activity was again remarkable (25-30%, $P<0.01$), however, there was a gradual fall in the percent elevation (about 20%). In the maintenance phase (24, 48,72 hr) the elevation in TAGH activity was persistent ($P<0.01$) which was followed by a fall in the percent elevation (about 20%) may be because of the 2/3 of Uranium which is excreted via kidney (13,16).

The elevation in the lipolytic activity under UN toxication can lead to various speculations.

(a) Since UN affects the cellular energetics under the toxic stress (5,7), there may be increase in energy demand and lipolysis must have been stimulated to produce the energy in the form of Free Fatty Acids (FFA).

(b) Under UN toxicity animal developes a "Fatty liver (5)" and in response to the accumulated lipids lipolysis must have been increased and FFA are released in the blood.

(c) The toxic shock developed by UN may cause release of adrenaline from adrenal medula which directly enhances the rate of lipolysis (17).

Thus, during UN acute toxicity, there is an overall increase in the rate of lipolysis of kidney, liver and adipose tissue. As a result of TAGH activity triglycerides are broken down and FFA liberated are released in the blood. This may cause the esterification of the free cholesterol to form cholesterol ester, the accumulation of which under such condition is evident (8). Blood being a transport medium, eventhough the TAGH activity was found to be elevated to considerable extent, this activity is not confined to the blood itself. The release of FFA from kidney, liver and adipose tissue (8) under such condition may manifest a false picture of elevation in the serum. Since in the present method, the release of FFA (as a result of TAGH activity) is an ultimate measure of lipolytic activity.

the elevation observed in serum could be because of the endogenous source of FFA from adipose tissue, kidney and liver.

Thus, the inter-relationship between lipemia and so called increase in TAGH activity of serum under UN intoxication is an intricate problem. Study of lipid metbolism and catabolism under such intoxication may give further information.

Conclusively, UN administration seems to stmulate the FFA release from adipose tissue, kidney and liver, with a rapid and significant increase in "TAGH" level which gives indication about the degree of intoxication and could be a possible diagnostic method. Further study in this regard is in progress in this laboratory.

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