A. OCHRACEUS TOXICITY ON CARBOHYDRATE METABOLISM IN CHICKS

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OCHRATOXIN producing fungi are ubiquitous and are reported to contaminate a wide variety of food and feed stuffs including black and red peppers, dry fish, rice, corn and peanuts[1-3]. Ochratoxin and ochratoxin-producing fungi have been isolated and purified from the food samples meant for human consumption in various parts of the world, including Canada, Japan and recently India. Reports reveal that *A. ochraceus* contamination is very frequent in India, especially in the southern region which is evident from the reported isolation and identification of ochratoxin in some of the warehouse samples in Tamilnadu and from the maize samples obtained from different poultry feed factories in Hyderabad[4,5].

Among the ochratoxins, ochratoxin A is a potent nephrotoxin which produces teratogenic effects in chick embryos[6]. Studies in this laboratory revealed drastic alterations in the cellular constituents in chicks, especially in carbohydrate metabolism during ochratoxicosis. In the present investigation an attempt has been made to study the key enzymes involved in carbohydrate metabolism and the blood glucose levels during toxicosis.

Yeast extract-sucrose medium suggested by Davis *et al*[7] was used for growing *A. ochraceus*. The strain was obtained from IARI, New Delhi. Ochratoxin A was extracted by the method of Steyn and van der Merwe[8] and purified using preparative "tlc". Authentic sample supplied by Dr Timothy D. Phillips, Department of Veterinary Public Health, Texas A and M University, was used as a reference.

The corn (250 g) was taken in Haffkin's flasks, the moisture content adjusted to about 40% and was sterilized in an autoclave at 15 psi pressure for 20 min. It was then inoculated with 1 ml of spore suspension containing 10^6 spores of *A. ochraceus* from a 12-day old culture. After incubation at 27°–30°C for 14 days, the organism was killed with chloroform and dried at 40°C for 5 days before grinding. The ground corn was mixed with normal chick mesh at 25% level and used as the experimental diet.

One-day-old chicks (from the Tamilnadu Poultry Research Station, Madras) were divided into three groups. The first group was fed with experimental diet. The isolated ochratoxin A was dissolved in 0.1 M aqueous sodium bicarbonate solution and 0.5 ml of the solution containing 10 μg of toxin was given intraperitoneally to another group of the experimental chicks, the control chicks were given normal chick mesh containing 25% corn.

After two weeks the surviving chicks were sacrificed and the blood samples were collected. The blood glucose was estimated by the method of Sasaki *et al*[9]. The liver, kidney and the intestine were removed immediately from the sacrificed chicks and homogenized in ice-cold Tris-HCl buffer (pH 6.4). The homogenates were used for the assay of enzymes and protein estimation[10]. Tissue aldolase and glucose-6-phosphatase were assayed by the method of King[11]. Fructose 1, 6-diphosphatase activity was assayed by an adaptation of the procedure described by Gancedo and Gancedo[12]. Brankstrup *et al*[13] method was employed for hexokinase assay.

Table 1 shows the blood glucose levels of normal and experimental chicks during ochratoxicosis. It is evident that blood glucose level gets elevated significantly in all the experimental birds indicating that this toxin may interfere in carbohydrate metabolism.

Table 2 shows the activities of hexokinase, aldolase, glucose-6-phosphatase and fructose 1,6-diphosphatase in control and experimental birds. The activities of hexokinase and aldolase, the insulin-dependent enzymes, are found to be reduced in experimental conditions.

Hexokinase which brings about the first phosphorylation step of glucose for metabolism is reduced significantly. This may be the reason for the diminished utilisation of glucose in the system and the increased amount of glucose in the blood. Further, the decreased activity of aldolase, one of the bifunctional enzymes of glycolysis, throws more light on the reduction of glucose oxidation in ochratoxin toxicosis through glycolysis. Toxins like patulin and penicillic acid[14,15] have been reported to react covalently with

Table 1 *Blood glucose levels of normal and (i) Ochratoxin A administered (ii) Contaminated diet of A. ochraceus administered chicks after 15 days treatment.*

Groups	Blood glucose
Control	82 ± 7.0
Ochratoxin A fed	128 ± 6.4 $P < 0.001$
Contaminated diet fed	116 ± 5.6 $P < 0.001$

Values are expressed as mg/dl of blood (Values are the average of five individual experiments in duplicate ± SD)

Table 2 *The levels of hexokinase, aldolase, glucose-6-phosphatase and fructose 1,6-diphosphatase in liver, kidney and intestine of control and experimental chicks.*

Enzyme	Organ	Control	Experimental Groups			
			Ochratoxin A fed		Contaminated diet fed.	
Hexokinase	Liver	4.8 ±0.38	3.1 ±0.21	$P<0.001$	3.7 ±0.28	$P<0.001$
	Kidney	9.6 ±1.04	6.4 ±0.75	$P<0.001$	7.9 ±0.62	$P<0.01$
	Intestine	15.7 ±1.65	10.8 ±0.80	$P<0.001$	12.1 ±1.54	$P<0.01$
Aldolase	Liver	14.2 ±1.30	9.9 ±0.76	$P<0.001$	10.7 ±0.81	$P<0.01$
	Kidney	5.1 ±0.47	3.8 ±0.27	$P<0.001$	4.1 ±0.39	$P<0.05$
	Intestine	14.1 ±1.50	10.6 ±0.78	$P<0.01$	12.0 ±1.20	$P<0.05$
Glucose-6-phosphatase	Liver	5.7 ±0.43	7.1 +0.51	$P<0.001$	6.8 +0.45	$P<0.01$
	Kidney	4.9 ±0.27	5.6 ±0.38	$P<0.01$	4.7 ±0.5	$P<0.01$
	Intestine	1.9 ±0.10	2.3 ±0.19	$P<0.01$	2.2 ±0.21	$P<0.05$
Fructose 1,6 di-phosphatase	Liver	0.82 ±0.06	0.98 ±0.08	$P<0.01$	0.93 ±0.07	$P<0.05$
	Kidney	0.36 ±0.02	0.42 ±0.03	$P<0.01$	0.40 ±0.03	$P<0.05$
	Intestine	0.72 ±0.07	0.84 ±0.06	$P<0.05$	0.78 ±0.05	NS

Values are expressed in μ moles of product formed/mg of protein. The values of six independent experiments with duplicate are expressed as mean ± S.D.

the -SH and also possibly with the $-NH_2$ groups of aldolase enzyme molecules which are essential for their activity. Ochratoxin A may also interact with the enzyme molecule in the similar way, resulting in the reduction of its activity.

The increased activities of FDPase and G-6-pase contribute to the enhanced gluconeogenesis in *A. ochraceus* toxicosis. Suzuki *et al*[16] reported that ochratoxin A decreased hepatic glycogen level with a marked decline in the glycogen synthetase activity and increased phosphorylase activity. The elevated blood sugar levels observed in the present investigation may be due to the combination of reduced glucose oxidation and glycogen synthesis and enhanced glyconeogenesis during ochratoxin toxicosis.

As the conditions of diabetes exist in *A. ochraceus* toxicosis, it appears that the toxin may act at a step thereby resulting in the reduction of insulin level in the system. The diabetogenic nature of the mycotoxin terreic acid has been reported by Shanmugasundaram *et al*[17]. The possibility of *A. ochraceus* toxicosis being diabetogenic, needs extensive work on their action, with pancreas and insulin molecule concentration.

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OCCURRENCE OF TODOROKITE FROM ADILABAD MANGANESE MINES, ANDHRA PRADESH

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TODOROKITE (Na, Ca, K, Mn^{2+}) (Mn^{4+}, Mn^{2+}, Mg)$_6$ O_{12}. $3H_2O$, a rare manganese rich mineral has been observed from the Adilabad Manganese Mines (Penganga Beds, Proterozoics), Andhra Pradesh (19° 39′ : 78° 40′). This is the only record of todorokite from India.

The area under investigation is Gollughat-Gunjala, Tamsi-Hattighat, Gotkur, Jamdapur and Metguda-Kanpa, a 32 km stretch with a discontinuous workable manganese body. The manganese ores occur in association with jasper, chert, shale, calcite and greyish-black limestone.

Ore microscopic studies of this deposit revealed the presence of psilomelane, pyrolusite, ramsdellite, groutite, hollandite, cryptomelane, braunite, birnessite and todorokite minerals. Todorokite is white in colour, fibrous and the reflectivity is higher than pyrolusite and cryptomelane. It shows uneven extinction under crossed nicols. Todorokite is found as replacement of birnessite, in the form of veins, which are parallel to jasper bands.

Electron microprobe analysis (Model Mar-2, USSR make, operating voltage 25 KV and specimen current 80–100) of todorokite shows Mn—69.11, CaO—2.00, Al_2O_3—1.57, Fe_2O_3—2.00, Na_2O—1.70, K_2O—0.65, MgO—0.33, Ni—0.05, Cu—0.31, Cr—0.12 and Co—0.10. X-ray analysis (Philips Rontgenerator, Cu K $\mathcal{L}$ radiation) of todorokite shows characteristic d-spacings of 9.576 Å (65), 4.786 Å (24) and 2.434 Å (16), which are correlative with Frondel *et al*[1], Straczeck *et al*[2] and Burns and Burns[3].

The manganese ores of Adilabad are the youngest, compared to the other manganese deposits of Sausers, Eastern Ghats and Dharwars[4,5]. Fromboidal manganese, also reported for the first time from this area[6], represents the presence of some primitive life activity, which might have caused the primary deposition of manganese at least in part[7].

Todorokite is found mostly in the recent marine basins and its occurrence is rare in continental deposits. The work is under progress in the delineation of the factors controlling the formation of todorokite.

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INFLUENCE OF HYDRATION-DEHYDRATION TREATMENTS OF RICE SEED ON THE GROWTH OF EMBRYO-ENDOSPERM TRANSPLANTS

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HYDRATION-DEHYDRATION treatment of stored seeds would invigorate them and the treated seeds would maintain vigour and viability for longer periods than untreated seeds[1]. Germinability of a cereal seed primarily depends on the embryo which is believed to deteriorate more rapidly than endosperm[2]. The degree of deterioration of the endosperm also influences the