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Increased Serum Corticosterone Levels in Triorthotolyl Phosphate-Treated Chickens

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Triorthotolyl phosphate (TOTP) has been described as the etiologic agent of "ginger paralysis", an ataxic syndrome suffered by an estimated 20,000 victims approximately 10 days after the consumption of an adulterated ginger-alcohol mixture (Smith et al. 1930). Subsequent studies have shown that the TOTP induced delayed neurotoxicity is produced via long axon degeneration and secondary demyelination (Cavanagh 1963). Peripheral sensory nerves are initially effected, followed by larger nerve degeneration, in particular the sciatic nerve (Cavanagh and MacDermot 1961).

Physiological changes which accompany axon degeneration in chickens treated with organophosphorus compounds that induce delayed neurotoxicity include weight loss, lipid metabolism changes (Joel et al. 1967), and reduced T-cell immunologic function (Foil et al. 1980). Increased levels of corticosterone, the predominant adrenal steroid of domestic fowl, have been shown to produce similar physiological changes in chickens (Nagra 1965; Sato and Glick, 1970). Additionally, vitamin E deficiency has been associated with TOTP induced delayed neurotoxicity (Draper et al. 1952) and increased corticosterone production (Rosenkrantz 1956). However, reports regarding the relationship of corticosterone and delayed neurotoxicity have conflicted. Ehrich and Gross (1982) reported a reduction in the clinical signs of delayed neurotoxicity in chickens fed corticosterone and subsequently treated with TOTP. Conversely, birds maintained in a low social stress environment (presumably with low plasma corticosterone) had less severe signs of TOTP induced neurotoxicity than birds maintained in a high social stress environment (Ehrich and Gross 1983).

The purpose of this study was to determine if there are changes in chicken serum corticosterone levels associated with TOTP induced manifestations. Additionally, the effects of orally or intraperitoneally administered vitamin E upon serum corticosterone levels and TOTP induced symptoms were examined.

MATERIALS AND METHODS

Adult female single comb white Leghorn chickens approximately 1 year old and weighing between 1.5 and 2.2 kg. were selected from a

Louisiana State University flock and randomly distributed into nine groups of 12 birds. The birds were placed in groups of three into standard 30.5 x 40.6 cm wire cages with food and water available ad libitum. The birds were adapted under these conditions for two weeks with diets of farm ration, farm ration + 50 mg/100g vitamin E, farm ration + 100 mg/100g vitamin E, or farm ration + 20 mg/100g metyrapone (2-methyl-1,2-di-3-pyridyl-1-propane, Sigma Chemical Co., St. Louis, MO). Farm ration was formulated to meet the minimum National Research Council (1977) requirements for laying hens. Vitamin E was incorporated at a level of 8.25 IU/kg finished feed. The birds remained on these diets until the experiment was terminated.

Triorthotolyl phosphate (TOTP) was obtained from Eastman Kodak Co., Rochester, New York, and orally administered in a 1:1 solution of triolein and TOTP at 1 ml/kg body weight (0.5 ml TOTP/kg). Twelve birds from each of the 4 test diets were treated with TOTP, and twelve birds from each test diet were administered 1 ml/kg triolein as controls. Additionally, intraperitoneal administration of 100 mg vitamin E was begun in one group of 12 birds maintained on farm ration at the time of TOTP treatment and continued daily throughout the experiment. The degree of ataxia (ataxic index) was graded from 0 (no effect) to 5 (total leg paralysis) similar to the method of Watanabe and Skarma (1977). Ataxic index was assigned by a researcher who had no knowledge of the individual bird's previous treatment. Bird weights were determined using an electronic balance on the day of treatment and 14 days later.

Serum corticosterone levels were sampled on the day of treatment and 14 days later using a radioimmunoassay. The techniques of this assay were the same as those described by Satterlee et al. (1980).

Absolute weight changes from day 0 to day 14, percent of total body weight changes, and plasma corticosterone measures were subjected to analysis of variance for treatment groups differences (Snedecor and Cochran 1967). Ataxic index on day 14 was treated as a nonparametric variable and analyzed by the procedures of Grizzle et al. (1969). Correlations made among weight change, ataxic index, and plasma corticosterone levels were biserial correlations.

RESULTS AND DISCUSSION

The nine groups of treated birds, Table 1, all originally contained 12 birds. However, deaths due to cardiac puncture on day 1 and other unrelated causes reduced the final number in 6 of the 9 groups. These deaths appeared to have little effect upon the results of the experiment.

The mean ± SD ataxic index on day 14 in the 5 groups treated with TOTP ranged from 1.4 ± 1.6 to 2.3 ± 1.1, Table 1. No treatment combined with or other than TOTP had any statistically significant effect upon the ataxic index on day 14. Weight loss associated with TOTP treatments is a consistent manifestation that is not entirely induced by lowered food consumption (Joel et al. 1967).

Weight losses between day 0 and day 14 in the TOTP treated groups ranged from 11.7 to 20.1% of total body weight. Weight changes in the groups receiving no TOTP ranged from a loss of 0.7% to a gain of 1.0% of total body weight, Table 1. No treatment combined with or other than TOTP had any statistically significant effect upon weight change between day 0 and day 14.

Plasma corticosterone levels on day 14 were significantly correlated to the ataxic index on day 14 ($r^2 = 0.48$, $p<.0003$) and weight loss between day 0 and day 14 ($r^2 = -0.43$, $p<.0013$). Additionally, ataxic index on day 14 and weight loss between day 0 and day 14 were significantly correlated ($r^2 = -0.556$, $p<.0001$).

Neither vitamin E nor metyrapone treatment had any effect upon the ataxia or weight loss induced by TOTP treatment. Vitamin E therapy has been shown to at least partially prevent or reverse the effects of TOTP treatment in species other than chickens (Draper et al. 1952: Hove 1953). Tissue vitamin E levels were not measured in this study; however, the lack of effects upon TOTP induced symptomology in chickens presented in this paper, supports the less detailed results previously reported by Casida et al. (1961).

Metyrapone has been shown to interfere with the enzymatic conversion of deoxycorticosterone (DOC) to corticosterone (Williamson and O'Donnell 1969), and prevent corticosteroid induced suppression of delayed-type hypersensitivity to sheep red blood cells in mice (Blecha et al. 1982). The RIA used in this study does not completely distinguish between DOC and corticosterone. Antiserum cross-reactivity with DOC has been estimated at 39.5% (Satterlee et al., 1980). While DOC is a known in vitro intermediate precursor of corticosterone (Wells and Wight, 1971), the presence of DOC in avian plasma has not been reported. However, metyrapone's assumed interference with the enzymatic conversion of DOC to corticosterone may have caused a buildup of DOC to levels sufficient for assay detection. If metyrapone treatment had affected weight loss or ataxia, a role of corticosterone in TOTP induced symptomology would have been indicated. However, the effects of metyrapone upon corticosterone production in chickens at the administered dosage was not demonstrated. Therefore, a role of plasma corticosterone level changes in TOTP induced symptomology cannot be excluded.

Increased serum corticosterone levels in chickens is generally considered to be an indicator of "stressed" conditions (Siegel 1980). Therefore, the increased corticosterone levels in TOTP treated birds could reflect a physically "stressed" response to the developing ataxia. Conversely, TOTP treatments could have caused serum corticosterone level increases by more direct mechanisms. Adrenocorticotropin hormone (ACTH) releases from the anterior pituitary or direct cortical adrenal stimulation of corticosterone production could be produced by TOTP metabolites. Additionally, decreases in tissue vitamin E levels (in particular adrenal cortical) can not be discounted as responsible for increased corticosterone production (Rosenkrantz 1956). Since TOTP has been shown to reduce gut absorption of vitamin E (Myers and Mulder

1953), adrenal vitamin E levels could have been depleted.

Regardless of the mechanism controlling increased corticosterone levels, corticosterone could contribute to TOTP induced manifestations. Weight loss, lipid metabolism changes, and depressed T-cell function have been described as symptoms of high serum corticosterone and TOTP administration. Therefore, there is a possibility that corticosterone increases contribute to some symptoms of TOTP treatment independent of the long axon degeneration that produces ataxia (Nagra 1965; Sato and Glick 1970).

There have been reports indicating that corticosterone levels could be directly involved in the development of ataxia. Cortisone treatment has been shown to be antagonistic to many of the effects produced by corticosterone treatment of chickens (Brown et al. 1958). Although the results have not been reproduced, Glees (1960) reported prevention of the onset of paralysis in TOTP treated chickens by the administration of cortisone three days prior to and every subsequent third day of TOTP treatment.

Ehrich and Gross (1983) indicated that low liver microsonal enzyme activities measured in low social stress birds could account for reduced clinical signs of delayed neuropathy. Presumably, low corticosterone levels are correlated with low MFO activity resulting in less metabolism of TOTP to the active neurotoxin, phenyl saligenin phosphate. This reported involvement of corticosterone is consistent with the data presented in this paper. Conversely, Ehrich and Gross (1982) reported reduced clinical signs of delayed neuropathy in chickens fed exogenous corticosterone. Due to the low number of experimental animals on which this 1982 report was based and the conflicting information subsequently presented, the effects of exogenous and endogenous corticosterone upon the clinical signs of TOTP treatment should be further investigated.

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Table 1. Mean total body weight changes between day 0 and day 14 and plasma corticosterone levels and ataxic index on day 14 of all treatment groups of hens

Treatment	N	kg weight change ± SD day 14 - day 0 (% total body wt. change)	Ataxic index day 14 ± SD	ng/ml plasma corticosterone day 14 ± SD	ng/ml plasma corticosterone day 14 - day 0
50 mg Vit E	11	-.013±.078(-0.7)	0	0.80± .40	+ .08
50 mg Vit E + TOTP	9	-.206±.125(11.7)	2.3±1.1	1.19± .87	+ .05
100 mg Vit E	12	+.014±.031(+0.8)	0	0.31± .25	- .36
100 mg Vit E + TOTP	10	-.249±.157(14.4)	1.7±0.8	2.23±2.07	+1.45
Farm Ration	11	+.007±.042(+0.4)	0	0.67± .44	- .03
Farm Ration + TOTP	10	-.368±.213(20.1)	2.2±1.3	1.73±1.11	+1.14
Farm Ration + TOTP + I.P. Vit E (100 mg)	12	-.265±.131(15.0)	1.4±1.6	1.92±1.47	+1.39
Metyrapone	11	+.019±.112(+1.0)	0	.58± .29	+ .04
Metyrapone + TOTP	12	-.278±.182(16.3)	2.2±1.2	1.93±2.54	+1.19

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