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Evaluation of Threonic Acid Toxicity in Small Animals

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ABSTRACT

Threonic acid, a breakdown product of additive ascorbic acid, was administered at a dietary level of 1% to male rats for 120 days and at levels of 0·05% and 0·2% to male and female mice until death occurred. There was no significant difference between the growth rates, organ weights, biochemical patterns and survival times of these test groups and similar control groups that had not received the acid.

INTRODUCTION

Ascorbic acid (L-xyloascorbic acid, vitamin C) is widely used as a technological aid in the food industry and the average consumer may ingest annually some 2–10 g of ascorbic acid breakdown products from additive sources (Thomas & Hughes, 1983). Of these breakdown products, threonic acid (threo-2,3,4-trihydroxybutyric acid) would appear to be of major significance and it has been detected, for example, in 'Chorleywood' process bread (Thewliss, 1971; Thomas, 1984) and in canned frankfurters (Thomas, 1984). Little is known of the dietary acceptability, or otherwise, of threonic acid, although earlier studies have indicated that it appeared to displace tissue ascorbic acid and to shorten the survival time of scorbutic guinea-pigs (Thomas & Hughes, 1983). This paper describes experiments designed to assess: (i) the influence, on male rats, of 1% dietary threonic acid, administered, as calcium threonate, for

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120 days, and (ii) the influence of dietary supplements of 0·05 % and 0·2 % threonic acid on the growth and survival time of male and female mice.

EXPERIMENTAL

Calcium threonate and diet

Calcium threonate was prepared by oxidation of ascorbic acid with hydrogen peroxide (Isbell & Frush, 1979) and characterised as previously described (Thomas & Hughes, 1983). Both the mice and rats received the synthetic diet previously described (Wright & Hughes, 1976) supplemented, for the test groups, with calcium threonate.

Experimental design

120-day toxicity study

Young male albino rats (Wistar) were housed in plastic cages at a room temperature of 22 °C and a relative humidity of 50 %. A control group (ten animals) received the casein–starch based synthetic diet; a test group (ten animals) received the synthetic diet containing 1 % calcium threonate. The diets were adjusted for isocaloricity (with starch) and calcium content (with calcium carbonate). Food intakes were measured at intervals during the experiment and body weights were recorded daily. After 120 days, the animals were killed by stunning and decapitation. Organs were freed from adventitious tissue, dried rapidly with filter paper and weighed within 5 min of their removal from the body. Blood was collected in heparinized tubes. Haemoglobin, packed cell volume, plasma cholesterol and cytochrome P-450 were determined by standard methods (Van Kampen & Zijlstra, 1961; Archer, 1965; van Roschlau *et al.*, 1974; Omura & Sato, 1964).

Life span study

Five-weeks-old mice (Tuck No. 1 strain) were randomly distributed between three groups and given the casein–starch synthetic diet; each group contained sixty-five males and sixty-five females. Group A was a control group, group B received a dietary supplement of 0·05 % calcium threonate, whilst group C received a supplement of 0·20 % calcium threonate. All animals received the appropriate diet until death occurred and their survival times were recorded.

RESULTS AND DISCUSSION

In both experiments there was no significant difference between the food consumed by the different groups. In the 120-day trial no differences were detected between the two groups, apart from a significant reduction in the relative liver weight in the test group (Table 1). In the life-span study, threonic acid at dietary concentrations of 0·05 % and 0·2 %, respectively, had no significant influence on the mean life-span of the mice (Table 2).

TABLE 1
The Effect of Dietary Calcium Threonate on Male Rats

	Calcium threonate in diet	
	0 %	*1 %*
Body weight (g): Initial	100·4 ± 1·4	103·4 ± 0·9
Final	286·4 ± 11·8	297·8 ± 13·6
Kidneys (g)[a]	1·87 ± 0·08	2·02 ± 0·09
% Body weight[b]	0·66 ± 0·01	0·68 ± 0·01
Liver (g)[a]	7·72 ± 0·37	7·37 ± 0·38
% Body weight[b]	2·71 ± 0·10	2·47 ± 0·04*
Spleen (g)[a]	0·394 ± 0·017	0·445 ± 0·022
% Body weight[b]	0·138 ± 0·005	0·143 ± 0·005
Adrenals (g)[a]	0·0287 ± 0·0012	0·0270 ± 0·0014
% Body weight[b]	0·0101 ± 0·0003	0·0092 ± 0·0006
Brain (g)[a]	1·95 ± 0·03	1·92 ± 0·04
% Body weight[b]	0·69 ± 0·03	0·65 ± 0·02
Blood		
PVC (%)	36·0 ± 1·8	38·7 ± 0·9
Haemoglobin (g/100 ml)	12·4 ± 0·6	12·8 ± 0·4
Plasma		
Cholesterol (mmol/litre)	1·96 ± 0·07	1·80 ± 0·15
Cytochrome P-450 (nmoles per gram of liver)	74·2 ± 12·7	57·50 ± 4·90

Values are means ± standard error for groups of ten rats; the experiment was of 120 days' duration. Values marked with an asterisk differ significantly from the control (* $P < 0·05$).

[a] Absolute weight.

[b] Relative weight.

TABLE 2
Influence of Threonic Acid (Supplements) on Mean Life-span (Weeks) of Male and Female Mice
(Means with standard error)

	Group		
	A	*B*	*C*
	Dietary calcium threonate		
	0%	*0·05%*	*0·20%*
Mean of group (males and females)	65·0 ± 2·0	62·8 ± 1·4	66·2 ± 2·2
Females	67·6 ± 3·2	66·2 ± 2·0	71·0 ± 2·8
Males	62·5 ± 2·3	59·5 ± 2·0	61·3 ± 3·2

Substantial amounts of 'additive' ascorbic acid are used annually in the United Kingdom as a technological aid in various food industries. Little of this additive ascorbic acid remains, *per se*, in the marketed product and the average consumer will therefore ingest substantial quantities of ascorbic acid breakdown products annually. It would appear, however, that to date very little work has been done to assess the nutritional acceptability of these breakdown products, despite indications that some of them may, under certain conditions, exercise a mutagenic effect (Hughes, 1981). Threonic acid is one of the better characterised breakdown products of additive ascorbic acid. It has, however, been shown to have no mutagenic effect in *Salmonella typhimurium* (Kalus *et al.*, 1982) and ascrobic acid itself proved negative in tests for primary mutagenicity carried out under conditions that would almost certainly have resulted in the formation of threonic acid (Ishidate *et al.*, 1984). Of other aldonic acids, gluconic acid has been widely used in an iron–sorbitol–gluconic acid complex for the treatment of anaemia (Fielding, 1977); reported adverse effects of calcium gluconate are apparently attributable to a direct toxicity of the calcium component (Book *et al.*, 1978). The animal studies described in this paper substantiate earlier findings and point to the essential lack of toxicity of threonic acid in mice and rats at dietary concentrations very much in excess of any amounts likely to be ingested by humankind.

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