GROWTH EFFECTS OF TIN COMPOUNDS IN RATS MAINTAINED IN A TRACE ELEMENT-CONTROLLED ENVIRONMENT[a]

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Received May 13, 1970

SUMMARY

In rats maintained on purified amino acid diets in trace element-controlled isolators, supplements of tin caused a significant growth effect. The diets were adequate in all known nutritional factors, including trace elements thus far identified as necessary, but they were composed of ingredients which kept trace element impurities at a minimum. Trimethyl tin hydroxide, dibutyl tin maleate, stannic sulfate, and potassium stannate enhanced growth at dose levels supplying 100 μg% (1 ppm) of tin to the diet. When supplied in form of stannic sulfate, 50, 100, and 200 μg% of tin increased growth by 24, 53, and 59%, respectively. These levels of tin are similar to those normally present in foods, feeds, and tissues. The results suggest that tin is a hitherto unrecognized essential trace element.

Trace amounts of tin occur widely distributed in tissues and nutrients but the element has been considered an "environmental contaminant" instead of an essential dietary trace factor (1). A relatively recent review on tin in man and foods, for instance, treated tin as an abnormal trace metal and concluded that "the evidence is convincing that measurable tin is not necessary for life or health" (2). This conclusion was based mainly on the fact that, with the prevailing, inadequate methods of analysis, "zero" levels of tin were found in the newborn and in organs of natives of some foreign countries. To our knowledge tin has indeed never been shown to play any physiological role in animals or man, and references to stimulatory activities in plants are found only occasionally in the literature (3, 4).

We have discovered that tin exerts a significant growth effect in the rat if trace element contamination from the environment is rigidly excluded.

[a]Supported in part by U. S. Public Health Service Grant AM-08669

EXPERIMENTAL

The growth experiments were carried out with weanling rats on purified amino acid diets, maintained in an ultraclean, trace element-sterile isolator system developed in our laboratory for the investigation of new trace element deficiency diseases (5, 6, 7). This technique makes it possible to grow small animals without direct contact with metal, glass, rubber, dust or caretaking personnel. The diets are based on an optimal mixture of L-amino acids and contain all chemically known nutritional factors in adequate and balanced amounts, including the nine trace elements identified thus far as being required. Results presented here were obtained with two diets, A and B. These diets are identical in their basic composition; they are modifications of rations described previously (5). Diet B constitutes an improvement over diet A with respect to freedom from trace element impurities. Differences between the diets are shown in Table 1. The tin compounds were added to the diets as freshly prepared aqueous suspensions.

Littermate, male, weanling Fisher 344 rats were divided among control and test groups. In the earlier experiments (diet A, 1968), each group consisted of five rats housed in the same cage. For experiments with diet B (1969/70), an improved isolator system was used which accommodates four groups of eight animals each in individual cages (8). As an additional control to each group inside the isolator, a group of six rats was raised on each of the test diets in metal cages under conventional conditions outside the isolator. Growth rates and appearance of the animals were evaluated at 3-4 day intervals The tests were terminated after 26-29 days. Growth rates and standard errors of the mean were computed by covariance analysis of the weight gains. Diets were analyzed for tin by atomic absorption spectroscopy (Perkin Elmer Model 303) after digestion either with nitric acid/perchloric acid or nitric acid/hydrogen peroxide mixtures.

RESULTS

Weanling rats maintained on the basal amino acid diet inside the trace

Table 1: Composition of Basal Diets

	%
Sucrose[a]	61.3 or 61.0
Lard[b]	10.0
Wesson Oil	5.0
Amino Acid Mixture S-7[c]	17.7
Fox Briggs Salts[c]	3.0
Trace Supplement[c]	0.1
Salt Supplement[d]	.73 or 1.05
Vitamin Mixture	1.0
Cellulose	2.0

a Diet A - Commercial sugar; Diet B - A.C.S. grade sucrose.
b Diet A - Canned stripped lard (Distillation Prod. Indus.,Rochester,NY); Diet B - Commercial packaged lard.
c See reference (6).
d Diet A - $NaHCO_3$, 0.112%; $NaHPO_4 \cdot H_2O$, 0.47%; K_2CO_3, 0.145%. Diet B - KH_2PO_4, Ultrex (J.T. Baker Chem. Co., Phillipsburg, NJ), 0.463%; Na_3 Citrate, 0.588%.

element-controlled isolator system show signs of deficiency within 1-2 weeks after initiation of the experiment (5). They grow poorly, lose hair, develop a seborrhea-like condition and are lacking in energy and tonicity. The condition is nutritional in origin since rats maintained on laboratory chow in the isolator are perfectly normal. The symptoms observed in the trace element-sterile environment are caused primarily by lack of hitherto unrecognized trace elements because supplements of 1% ash from yeast largely, but not entirely, prevent their occurrence. Chemical fractionation of yeast ash has provided evidence for the involvement of more than one element in the prevention of the deficiency symptoms (8).

The possibility exists that various tin derivatives could differ widely in activity, in analogy to selenium (9) and chromium (10). The number of compounds suitable for experimentation is limited since many tin compounds, especially the organo-tin derivatives, are either insoluable in water or unstable. The 2+ oxidation state is readily oxidized by air to the 4+ state.

Table 2: Growth Effect of Tin Compounds in Rats in Trace Element-Controlled Environment
(Duration of experiments 26-29 days)

Compounds	Dose Level μg Sn/100g	No. of Animals	Average Daily Weight Gain g	Increase %	P Value
Basal A					
Control	-	5	1.89 ± 0.06[a]	-	-
Trimethyl tin hydroxide, $(CH_3)_3SnOH$	100	4	2.22 ± 0.09	18	.02
Dibutyl tin maleate, $(C_4H_9)_2Sn(OOCCH=)_2$	100	5	2.16 ± 0.14	14	.1
Basal B					
Control	-	7	1.27 ± 0.11	-	-
Stannic sulfate, $Sn(SO_4)_2 \cdot 2H_2O$	100	7	1.67 ± 0.07	31	.01
Potassium stannate, $K_2SnO_3 \cdot 3H_2O$[b]	100	8	1.55 ± 0.10	22	.1
Control	-	5[c]	1.10 ± 0.05	-	-
Stannic sulfate, $Sn(SO_4)_2 \cdot 2H_2O$	50	8	1.37 ± 0.10	24	.02
" "	100	8	1.68 ± 0.10	53	$<.001$
" "	200	8	1.75 ± 0.10	59	$<.001$

[a] Mean ± standard error.
[b] Better formulated as $K_2[Sn(OH)_6]$.
[c] Two control animals died in the course of the experiment. One animal was eliminated because it was outside of the normal error.

Therefore, the current studies were carried out with tetravalent compounds which are soluble in water and relatively stable in solution. Both inorganic salts and organic derivatives of tin were found effective.

Results obtained with tin supplements are presented in Table 2. With diet A, positive effects are shown with two organo-tin compounds, trimethyl tin hydroxide and dibutyl tin maleate, each used at dose levels supplying 100 μg of tin per 100 g of diet (1 ppm). While the result with trimethyl tin hydroxide was statistically highly significant, that with dibutyl tin maleate was significant only at 90% level.[b] The results with the improved diet B illustrate the effectiveness of inorganic tin compounds. Stannic sulfate produced sizeable growth effects while potassium stannate elicited a lesser response. However, more data are needed to clarify whether the hexa-aquo complex of tin in potassium stannate is truly less effective than tin in stannic sulfate. The latter compound is extensively hydrolyzed in water (11). When stannic sulfate was employed at varying levels (last experiment, Table 2) a dose response curve was obtained. With 50 μg% of tin (.5 ppm) the effect was approximately half as great as with 100 μg%. The latter level was nearly optimal, since 200 μg% produced a similar, not significantly greater response.[c]

While the tin supplements promoted growth, they did not fully cover the missing requirements of animals inside the isolator system. In all experiments shown on Table 2, tin affected the hair and skin changes only to a minor degree. A comparison of the weight gains attained inside the isolator system with those outside showed that unsupplemented inside animals grew only approx-

b Five other successive experiments with trimethyl tin hydroxide using a modification of diet A have been carried out. Three produced positive, significant responses while two others showed no effect. Lack of effect was correlated to high growth rates of the unsupplemented controls and to contamination of the basal diet with tin. Emission spectrography revealed tin levels varying from "non-detectable" to 13 ppm. A batch of diet producing high growth rates in unsupplemented controls and showing no response to tin supplements was found by atomic absorption to contain 6.4 ppm of the element.

c Numerous other elements have been tested and were found to be inactive.

imately 40-45% as fast as their outside controls (Table 3). Supplements of tin did not enhance the growth of animals under conventional conditions. Rats receiving tin supplements in the isolator reached 60% of the growth rate of their conventional controls, which confirms that more than one element is involved in the prevention of the impaired growth rates in the trace element-sterile environment. Identification of the other missing element(s) is under way.

Table 3: Comparison of Growth Rates Inside Trace Element Sterile Isolator to Outside Controls in Conventional Caging[a]

Compounds	Dose Level	Average Daily Weight Gain		Ratio[b]
		Isolator	Conventional	
	µg Sn/100g	g	g	
Control	-	1.32 ± 0.09[c]	2.96 ± 0.18	.445
Stannic sulfate	100	1.67 ± 0.07	2.79 ± 0.09	.600
Potassium stannate	100	1.55 ± 0.10	2.89 ± 0.14	.536
Control	-	1.10 ± 0.05	2.69 ± 0.10	.409
Stannic sulfate	50	1.37 ± 0.10	2.97 ± 0.08	.462
" "	100	1.68 ± 0.10	2.60 ± 0.07	.647
" "	200	1.75 ± 0.10	2.92 ± 0.11	.600

a Experiments with diet B from Table 2.
b Ultraclean/conventional.
c Mean ± S.E.

DISCUSSION

The levels of tin found to be effective in the promotion of growth under our experimental conditions are quite similar to those found in almost all foods of plant or animal origin and in tissues (2, 12, 13). They are also similar to levels of tin in human organs. Reports on the presence of tin in tissues, including embryos, go back half a century (14). For instance, in human kidney, liver, and lung from different geographical areas, mean concentrations of 23-70, 35-100, and 44-120 µg% of tin were reported on a wet weight basis (2). However, the accuracy of these values depends on the sensitivity of analytical methods applied and on the preparation of the sample

Most organic tin derivatives evaporate below 200°C, and even inorganic tin salts, such as stannic chloride and stannic acetate, have low boiling points. Nevertheless, most of the values for tin in tissues and nutrients in the literature are based on determinations carried out after drying of the specimens at 110°C and subsequent ashing at 450°C. Under these conditions serious losses of tin may occur. The chemical nature of the tin compounds in biological specimens is unknown.

Tin belongs, with carbon, silicon, germanium, and lead, to the IVth main group of elements. It has a number of chemical properties which offer interesting possibilities for biological functions. Tetravalent tin has a strong tendency to form coordination complexes with 4, 5, 6, and possibly 8 ligands. Thus it could contribute to the tertiary structure of proteins or other components of biological importance. Tin also forms covalent bonds with carbon and a large number of organo-tin compounds are known. Some of these serve as catalysts in polymerization, transesterification, olefin condensation, and other reactions. Furthermore, tin participates in oxidation-reduction reactions. The $Sn^{2+} \rightleftharpoons Sn^{4+}$ potential is at -.13 V, i.e., well within the physiological range. It is close to the oxidation-reduction potential of flavine enzymes.

It is noteworthy that most tin compounds are of low toxicity and that the element is not progressively accumulated in the organism even though the daily intake in man has been calculated to be 3.6 mg on an institutional diet (2). Others have estimated intakes of 17 mg or more (12). Since the animals in our experiments, weighing 30-100 g, eat approximately 5-10 g of diet per day, the effective dose level of 100 µg% affords a daily intake of 5-10 µg of tin per animal.

The results indicate that tin may be an essential trace element for the mammalian organism.

Acknowledgments: The effective technical assistance of David W. Evans, Maureen Conley, George F. Collins and George S. El-Bogdadi is gratefully acknowledged.

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