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A Study of the Effects of Zinc and Tin Administered Orally to Mice over a Prolonged Period

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Abstract—Mice which received 1000 or 5000 ppm tin as sodium chlorostannate in drinking water or 5000 ppm tin as stannous oleate in their diet experienced a lower incidence of malignant lymphoma, hepatoma and pulmonary adenoma than untreated controls, and showed no other untoward effects.

Mice which received 5000 ppm zinc as zinc oleate in the diet developed severe anaemia. Accordingly the level of zinc oleate was reduced to 1250 ppm. Prolonged feeding at this level failed to increase the incidence of malignant lymphoma or pulmonary adenomata above control levels. A slight but probably insignificant increase of hepatomata was recorded. The inclusion of 1000 or 5000 ppm zinc as zinc sulphate in the drinking water did not lead to an increased incidence of tumours at any site.

INTRODUCTION

There have been few long-term toxicity or carcinogenicity tests of inorganic tin or zinc salts, despite the occurrence of these metals as contaminants in food which has been canned or stored in galvanized containers. It was because of the lack of adequate chronic toxicological studies and because canned food had been indiscriminately accused of causing cancer (House of Lords, 1961) that the experiments described below were undertaken.

EXPERIMENTAL

Materials. Sodium chlorostannate, $Na_2SnCl_6.5H_2O$ (technical grade), stannous oleate $(C_{17}H_{33}COO)_2Sn$ (technical grade), zinc sulphate $ZnSO_4.7H_2O$ (Analar grade) and zinc oleate $(C_{17}H_{33}COO)_2Zn$ (technical grade) were obtained from British Drug Houses Ltd. (Poole, Dorset).

Animals. Chester Beatty stock mice were used. During the experiment they were housed in metal cages, 4–6 per cage.

Basic diet. The basic diet fed to all groups was prepared from a meal-mix supplied by Messrs. Dixon, of Ware, Herts., to which "Bemax" stabilized wheat germ (Vitamins Ltd., Crawley, Sussex), arachis oil (*British Pharmacopoeia* Standard, from Savory and Moore Ltd., London) and Vitamin A and D concentrates (British Drug Houses Ltd., Poole, Dorset) were added. The basic diet had the following percentage composition: meal-mix (wheat flour, 68·7; casein, 11·4; milk powder, 8·0; chalk, 1·3; salt mixture, 0·8; and yeast, 2·3); "Bemax" stabilized wheat germ containing carbohydrates, protein, B vitamins, manganese, iron, copper, essential amino acids, 2·5; arachis oil and vitamins A (40,000 I.U.) and D (4000 I.U.) concentrate 5·0. Zinc and tin salts were mixed into the diets of groups 3 and 6 (*vide infra*). Enough tap water was added to the dry diets to make a dough which was fed to the mice *ad lib.*

Test diets

Additions to basic diet. Stannous oleate (31·25 g/kg) and zinc oleate were added to the basic diet and fed to groups 3 and 6 (including 6W) respectively. The level of zinc oleate began at 50 g/kg basic diet, but was reduced after 3 months to 25 g/kg, and to 12·5 g/kg after a further 3 months, because of deaths from anaemia.

Additions to drinking water. Sodium chlorostannate was dissolved in distilled water and given as drinking water to groups 1 and 1W (15 g/l) and to groups 2 and 2W (3 g/l). It was prepared freshly each day because of a slow-forming gelatinous precipitate. Groups 4 and 5 received respectively 22 g and 4·4 g zinc sulphate/l of distilled water as drinking water. Groups 3, 6, 6W, 7 and 7W were given tap water.

Experimental design and conduct. Newly-born litters were allotted randomly to the 7 dietary groups (groups 1–7) shown in Table 1. The lactating mothers and sucklings received the diets up until the time the young were weaned. When the young mice were removed from their mothers they were numbered on the ears and rehoused in boxes of 4–6 according to group and sex. Thereafter they continued to receive the same treatment as they had before weaning.

Table 1. *Dosage schedule of zinc and tin in mice*

Group	Diet	Drinking water
1 and 1W*	Basic diet	15 g sodium chlorostannate/l distilled water (5000 ppm Sn)
2 and 2W	Basic diet	3 g sodium chlorostannate/l distilled water (1000 ppm Sn)
3	31·25 g stannous oleate/kg basic diet (5000 ppm Sn)	Tap water
4	Basic diet	22 g zinc sulphate/l distilled water (5000 ppm Zn)
5	Basic diet	4·4 g zinc sulphate/l distilled water (1000 ppm Zn)
6 and 6W	50 g (which was reduced to 25 g then to 12·5 g) zinc oleate/kg basic diet (5000 ppm Zn for 3 months; 2500 ppm for 3 months; then 1250 ppm until end of experiment)	Tap water
7 and 7W	Basic diet	Tap water

*Groups 1W, 2W, 6W and 7W were introduced during experiment following heavy losses of mice in first 8 weeks caused by an epizootic of ectromelia.

An epizootic of ectromelia in the mouse colony caused the deaths of several mice during the first 8 weeks of the experiment. The survivors were vaccinated with sheep lymph and any animal which showed a negative or accelerated response was killed. Many apparently healthy mice were sacrificed for this reason. Because numbers were so depleted new groups of weanling mice (4–5 weeks of age) were set up to supplement groups 1, 2, 6 and 7. These supplementary groups are shown in Tables 1 and 2 as groups 1W, 2W, 6W and 7W. Weanlings were used at this stage because they were available and newly-born litters were not.

The mice were examined thoroughly once each week throughout the experiment and more cursorily each day when they were fed. They were weighed once every 2 weeks. There was no appreciable difference in the rates of gain of body weight between test and control groups, except that mice in group 6 which became anaemic during treatment were severely stunted. Surviving mice were killed 1 yr after the start of treatment.

All mice which died during the experiment or which were killed at the end of the experiment were subjected to a thorough post-mortem examination. Lesions which were probably or possibly neoplastic were taken for histological examination. Stomachs were routinely distended with formol saline. After fixation they were opened and examined macroscopically and microscopically for tumours and other changes in the forestomach and glandular epithelium.

RESULTS

In all groups most of the deaths which occurred during the first 8 weeks of the experiment were due, directly or indirectly, to ectromelia. There was excessive mortality in group 6. At post-mortem examination most of the mice in this group showed marked anaemia. Blood from the orbital sinus of 4 mice selected at random from group 6 had a haemoglobin content of only 40% of the control level. The packed cell volume of the blood was correspondingly low. Approximately 17 of the 30 deaths in group 6 between the time of weaning (week 4) and week 15 of the experiment were due to anaemia.

Table 2 shows the incidence of tumours of all sites in mice which survived for 45 weeks or more. The findings in animals which died before week 45 of the experiment followed a similar pattern. There was no sex difference in tumour incidence either before or after week 45.

Table 2. *Incidence and types of tumours in mice surviving 45 weeks of zinc or tin treatments*

Group	No. of mice killed during weeks 45–53	No. of mice with*		
		Hepatoma	Malignant lymphoma	Lung adenoma
		TIN		
1	8	–	1	2
1W†	5	–	–	1
2	9	1	–	3
2W	7	1	1	–
3	30	5	1	13
Total....	59	7(11·9%)	3(5·1%)	19(32·2%)
		ZINC		
4	22	3	2	5
5	28	3	4	9
6	11	1	1	5
6W	12	6	1	4
Total....	73	13(17·8%)	8(11·0%)	23(31·5%)
		CONTROL		
7	19	1	2	8
7W	5	2	1	2
Total....	24	3(12·5%)	3(12·5%)	10(41·7%)

*No other tumours developed with the exception of 1 haemangioma (subcutaneous) in group 2.
†See footnote to Table 1.

Sections of forestomach epithelium from mice in different groups were examined for evidence of hyperplasia. No differences between any of the treated or control groups were observed.

DISCUSSION

The toxicology of zinc and tin has been fully reviewed by Browning (1961). The signs of acute zinc poisoning in animals (dogs, rats, mice and guinea-pigs) are enteritis, tremors and paralysis; the less acute effects are lack of growth and anaemia. Heller & Burke (1927) found that 2500 ppm zinc, as zinc chloride or zinc carbonate in the diet, was without effect on rats, but in experiments reported by Sutton & Nelson (1937) and Smith & Larson (1946) zinc, at the level of 10,000 ppm, caused anaemia in rats when fed as zinc carbonate in the diet.The acute toxicity of zinc to the rat was shown by McCall, Mason & Davis (1961) to be affected by the source and level of the dietary protein.The only toxic effect observed in the present experiment was anaemia in the mice fed zinc oleate at levels of 2500 and 5000 ppm of zinc.

Outbreaks of acute poisoning attributable to the preparation or storage of food in galvanised containers have been reported in the *British Medical Journal* (1923) and by Callender & Gentzkow (1937), Dornickx (1938) and Brown, Thom, Orth, Cova & Juarez (1964). The zinc content of the food was of the order of 800 ppm and the acute symptoms were nausea, vomiting and diarrhoea. There are no records of chronic poisoning.

With regard to the carcinogenicity of zinc, the results reported in this paper do not confirm those of Halme (1961). He claimed a high tumour incidence in both tumour-resistant and tumour-susceptible strains of mice given from 1 to 200 mg of zinc/l (i.e. 1–200 ppm) of drinking water. Halme (1961) did not state in which form the zinc was administered. Only in one case in the present experiment did the incidence of tumours in a test group exceed that of the controls: 7 out of 23 mice in groups 6 and 6W (zinc oleate in diet) developed hepatomata, as compared with 3 out of 24 controls. The difference is probably not significant.

Zinc salts induce teratoma of the testis in fowls (Guthrie, 1956 & 1964) and, in combination with various hormone treatments, testicular tumours in rats (Rivière, Chouroulinkov & Guérin, 1959). No testicular tumours were observed in the present experiment.

The levels of zinc tested were greatly in excess of the United Kingdom recommended levels in food (50 ppm), beverages (5 ppm) and edible gelatin (100 ppm) (Food Standards Committee, 1953). Higher levels of zinc may occur naturally in some animal and vegetable products, e.g. herrings, shell-fish, crustacea and cereal and animal offals, and after the storage of food, particularly acid and saline liquids in galvanized containers, which has been shown to cause acute poisoning. There is no evidence from these experiments that zinc at any level in food is likely to be carcinogenic.

According to Browning (1961), large doses of inorganic tin cause injury to the central nervous system and gastro-intestinal disturbance. Haddon (1958) found that rats injected intraperitoneally with stannous chloride developed the classical lower-nephron lesions of heavy metal poisoning. Organic tin compounds may produce cerebral oedema, paralysis, skin irritation and inflammation of the bile ducts (Barnes & Magee, 1958; Barnes & Stoner 1958). No toxic effects were observed in mice treated with tin in the present experiment.

There are no reports in the scientific literature to suggest that tin is carcinogenic. Even the implantation into the subcutaneous tissues of rats of tin foil failed to evoke the appearance of tumours, whereas similar subcutaneous implants of several other metals gave rise to sarcomata (Oppenheimer, Oppenheimer, Danishefsky & Stout, 1956). The results of the present experiment provide further support for the view that tin is safe from the point of view of carcinogenesis.

Up to 1953 the maximum permitted level of tin in canned food in the United Kingdom was 286 ppm. This recommendation, made in 1908, was based not on the results of biological research but on the fact that levels rarely exceeded this during routine canning of a variety of food products (Food Standards Committee, 1952). In 1953 the permitted maximum was reduced to 250 ppm (Ministry of Agriculture, Fisheries and Food Trade Press Notice No. 1553T) "since a high tin content in food would be contrary to good commercial practice". Before the introduction of lacquering, the tin content frequently rose during prolonged storage to levels much in excess of the tolerated maximum (Monier-Williams, 1950) but nowadays the tin content of food in unopened cans rarely rises above 40 ppm (Adam & Horner, 1937). The tin content of food left in an opened can may rise as a result of oxidative processes (Glassmann & Barzutskaya, 1928). The levels of tin employed in these long-term tests were greatly in excess of those likely to occur in canned food. There was no evidence of chronic toxicity nor of the development of tumours in the mice as a result of the treatment.

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Etude des Effets du Zinc et de l'Etain Administrés Oralement á des Souris Pendant une Période Prolongée

Résumé—Des souris qui reçurent 1000 ou 5000 ppm d'étain sous forme de chlorostannate de sodium dans leur eau de boisson ou 5000 ppm d'étain sous forme d'oléate stanneux dans leurs aliments présentèrent une tendance moins marquée aux lymphomes, hépatomes et adénomes pulmonaires malins que celles qui ne reçurent aucun traitement, et ne manifestèrent aucun autre effet nocif.

Chez des souris qui reçurent 5000 ppm de zinc sous forme d'oléate de zinc dans les aliments, il se produisit une sévère anémie. En conséquence on réduisit la dose de zinc à 1250 ppm. La prise prolongée de zinc à cette dose n'éleva pas la proportion des lymphomes malins au-dessus du niveau trouvé chez les témoins. On nota une légère augmentation du nombre des hépatomes, vraisemblablement sans signification. L'addition de 1000 ou 5000 ppm de zinc, sous forme de sulfate, à l'eau de boisson n'entraîna la formation de tumeurs en aucun point de l'organisme.

Untersuchung der Wirkung Langzeitig Oral an Mäuse Verabreichten Zinks und Zinns

Zusammenfassung—An Mäusen, die 1000 oder 5000 ppm Zinn als Natriumchlorstannat im Trinkwasser oder 5000 ppm Zinn als Zinn(II)-oleat im Futter erhielten, wurde ein im Vergleich zu unbehandelten Kontrolltieren vermindertes Auftreten maligner Lymphome, Hepatome und pulmonaler Adenome ohne ungünstige Nebenwirkungen beobachtet.

Bei Mäusen, die 5000 ppm Zink als Zinkoleat im Futter erhielten, entwickelte sich schwere Anämie. Der Gehalt an Zinkoleat wurde deshalb auf 1250 ppm vermindert. Die längere Verabreichung dieser Dosis bewirkte keine grössere. Häufigkeit maligner Lymphome und pulmonaler Adenome im Vergleich zu den Kontrolltieren. Eine geringe, aber wahrscheinlich nicht signifikante Zunahme der Hepatome wurde beobachtet. Die Verabreichung von 1000 oder 5000 ppm Zink als Zinksulfat im Trinkwasser führte nicht zu gehäuftem Auftreten von Tumoren in irgendeiner Körperregion.

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