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STANNOUS CHLORIDE

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SUMMARY

Description

Stannous chloride is a white or colorless solid, with a slight odor of hydrochloric acid. It is very soluble in water, and at low concentrations forms a basic salt that is oxidized to basic stannic salt in the presence of air.

Occurrence

Stannous chloride as a food additive is analogous to the mineral tin to which man is exposed as a natural food contaminant. As a food additive, up to 20 ppm may be incorporated in a given food, (032), with an average estimated daily intake from all food sources of 4 mg (032, 124). Small amounts also occur in sea water, city water, and in air in urban regions.

Acute Toxicity

An LD_{50} of 215 mg/kg body weight has been reported for mice (111), and for a dog LD 200 mg/kg BW (025), both by the oral route. An outbreak of human gastrointestinal distress was observed in 38 women drinking punch containing 2000 mg tin per liter of punch (ca. 2000 ppm) (151).

Short-Term Studies

Intramuscular doses of 60 mg/kg/day for 6 days (a total dosage of 360 mg) proved lethal to mice, while 4 mg/kg/day for 8 days was apparently well tolerated (025). By the same route, a total toxic dose of 240 mg/kg administered as 40 mg/kg/day, was recorded for guinea pigs and rabbits. All animals showed pathological lesions in the lungs, liver, and kidneys, and tin deposits in the last two organs.

Long-Term Studies

Mice and rats given 5 µg/ml Sn, as stannous chloride, in their drinking water, evidenced a slight alteration in median life-span and in longevity (125,126). For males and females the life-span was the same, and the longevity of males greater than females. This is unusual, as normally females of both species live longer than males.

Also, the body weight of male rats was lowered, and there was accumulation of small amounts of tin in the tissues.

Biochemistry

It has been shown that tin accumulates in the tissues of mice (125), rats (126), and rabbits (142). Also, man accumulates it in amounts varying among individuals, geographical location (124), and industrial background (063). However, studies on urinary and fecal excretion by man (017,063) have given results indicating that the human organism establishes a balance, excreting in the feces tin values practically equivalent to the daily intake of the substance.

Carcinogenicity

In mice given 5 µg/ml stannous chloride in their drinking water (estimated intake 35 µg/100 g/day), (062) it was noted that both the number and the types of tumors found in the treated animals corresponded to those of the untreated controls, and also the ratio of malignant to benign tumors was essentially the same in both groups of animals. In the case of rats given the same dose (126) there actually was a drop in percentage of tumor-bearing animals from that of controls; 37.8% for the latter compared with 29.9% for the former. Also, in mice given sodium chlorostannate (tetravalent tin, 1000 or 5000 ppm in drinking water), a lower incident of malignant lymphoma, hepatoma, and pulmonary adenoma than in the controls was noted. In 30 inbred August hooded rats fed 2% chlorostannate (117) in the diet for a year, 3 malignant tumors were found; in 27 rats fed first 1% then 0.5% stannous 2-ethyl hexoate, no neoplasms were found; and in 33 untreated controls no malignancies were noted. The author considers these results promising, but not definitive.

Teratology

Albino CD-1 outbred mice, albino Wistar rats, and golden hamsters were fed up to 50 mg/kg/day of stannous chloride by oral intubation (031) with no "clearly discernible effect on nidation, maternal or fetal survival, or on

abnormalities in soft or skeletal tissues." Likewise, stannous chloride was found to have no teratologic effect on chick embryos (116) by the particular experimental procedure attempted. A human case (106) of right-hand agenesis occurred after the ingestion of pharmaceutical doses of 60 mg pure tin oxide plus pure tin, 3 tablets per day for 5 days. However, the dose level is so much greater than routinely encountered in foods that the results are not comparable.

Safe Levels

On the basis of the data assembled here, it would appear that the threshold value for acute intestinal distress occurs between ca. 200 ppm (an average high value noted for normally ingested foods) (011,124) and the 2000 ppm in the punch which caused the outbreak in 38 women (151). Although the WHO/FAO 1972 report (035) recommends more experimental study, no documents reviewed recommended modification of the present regulation of a maximum of 20 ppm of the food additive in a food stored in an inert container.

CHEMICAL INFORMATION

I. Nomenclature

A. Common name: Stannous chloride

B. Chemical name: Stannous chloride dihydrate

C. Trade names: Stannous chloride, tin (II) chloride dihydrate, Stannochlor

D. Chemical Abstracts Services Unique Registry Number: 10025691

II. Empirical Formula

$SnCl_2 \cdot 2H_2O$

III. Structural Formula

$SnCl_2 \cdot 2H_2O$

IV. Molecular Weight: 225.63 (the usual dihydrate form)

V. Specifications

A. Technical Grade (data supplied by FDA)

Assay ($SnCl_2 \cdot 2H_2O$)	96.0%
Solution in HCl	Passes test
Other Metals (as Pb)	0.04%

B. Reagent grade, A.C.S. specifications:

Assay ($SnCl_2 \cdot 2H_2O$)	98.0-102.0%
Solution in HCl	passes A.C.S. test
Sulfate (SO_4)	$<$0.003%
Arsenic (As)	0.0002%
Substances not precipitated by H_2S (as SO_4)	0.050%
Iron (Fe)	0.003%
Other metals (as Pb)	0.010%

C. Food grade, as specified by the M&T Chemicals Inc. (American Can Co.), Rahway, N.J.

Stannochlor (Food Grade)

Stannous		62.2%	
Iron	(Fe)	0.010%	maximum
Antimony	(Sb)	0.0015%	maximum
Sulfur	(S)	0.0001%	maximum
Arsenic	(As)	0.0006%	maximum
Lead	(Pb)	0.005%	maximum
Copper	(Cu)	0.001%	maximum
Insoluble		0.02%	maximum

D. Food Chemicals Codex, 1972 Edition (022), presents the following specifications for stannous chloride:

1. Description

White or colorless crystals having no odor or a slight odor of hydrochloric acid. It is very soluble in water and is soluble in alcohol and in glacial acetic acid.

2. Identification

A. To a 1 in 20 solution of the sample in diluted hydrochloric acid T.S. add mercuric chloride T.S. dropwise. A white or grayish white precipitate is produced.

B. A 1 in 20 solution of the sample gives positive tests for chloride.

3. Specifications

Assay. Not less than 98.0% and not more than the equivalent of 102.2% of $SnCl_2 \cdot 2H_2O$.

Solubility in hydrochloric acid. Passes test.

Limits of Impurities

Arsenic (as As). Not more than 3 ppm (0.0003%).

Other heavy metals (as Pb). Not more than 100 ppm (0.01%).

Iron. Not more than 30 ppm (0.003%).

Substances not precipitated by sulfide. Not more than 0.05%.

Sulfate. Passes test (about 0.003%).

4. Tests

Assay. Transfer about 2 g of the sample, accurately weighed, into a 250-ml volumetric flask, dissolve in 15 ml of hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a 500-ml flask, add 5 g of sodium potassium tartrate, and mix. Make the solution alkaline to litmus with a cold saturated solution of sodium bicarbonate, and titrate at once with 0.1 N iodine, using starch T.S. as the indicator. Each ml of 0.1 N iodine is equivalent to 11.28 mg of $SnCl_2 \cdot 2H_2O$.

Solubility in hydrochloric acid. A 5-g portion of the sample dissolves completely in a mixture of 5 ml of hydrochloric acid and 5 ml of water, heating to 40°, if necessary, to effect solution.

Arsenic. Determine as directed under Arsenic Test, modifying the Procedure as follows: Mix 1.0 g of the sample in the generator flask with 35 ml of water and 4 g of 20-mesh zinc. Add 20 ml of dilute sulfuric acid (1 in 5), but omit the potassium iodide and stannous chloride reagent solutions. Immediately connect the generator flask to the lead acetate cotton scrubber unit and the absorber tube containing 3.0 ml of silver diethyldithiocarbamate solution, and allow the reaction to continue for 45 minutes as directed in the Procedure.

Other heavy metals. Dissolve 1 g of the sample in a mixture of 2 ml of hydrochloric acid and 3 ml of nitric acid, and boil until solution is complete and brown fumes are no longer evolved. Cool, and dilute to 50 ml with water. To 10 ml of this solution add 8 ml of sodium hydroxide solution (1 in 10), then cool, and dilute to 40 ml with water. Prepare a control containing 2.0 ml of standard lead solution (20 µg Pb), 8 ml of the sodium hydroxide solution, and 30 ml of water. Add 10 ml of hydrogen sulfide T.S. to each solution. Any color produced in the solution of the sample does not exceed that in the control.

Iron. Add 3 ml of dilute hydrochloric acid (1 in 2) to the residue obtained in the test for substances not precipitated by sulfide, cover with a watch glass, and digest on a steam bath for 15 minutes. Remove the cover, and evaporate to dryness on the steam bath. Dissolve the residue in a few ml of water and 8 ml of hydrochloric acid, dilute to 100 ml with water, and mix. To 25 ml of this solution add 25 ml of water, 400 mg of ammonium persulfate crystals, and 3 ml of ammonium thiocyanate T.S. Any red or pink color does not exceed that produced by 1.5 ml of iron standard solution (15 µg of Fe) in an equal volume of solution containing the quantities of the reagents used in the test.

Substances not precipitated by sulfide. Dissolve 4 g of the sample in 5 ml of hydrochloric acid, dilute to 200 ml with water, and pass hydrogen sulfide through the solution to completely precipitate the tin. Filter without washing, and evaporate 100 ml of the filtrate to a few ml in a tared dish. To the residue add 0.1-0.2 ml of sulfuric acid, and evaporate to dryness. Ignite at 800 ± 25° for 13 minutes, cool, and weigh. Retain the residue for the Iron test.

Sulfate. Dissolve 5 g of the sample in 5 ml of hydrochloric acid, dilute to 50 ml with water, filter if not clear and heat the filtrate or clear solution to boiling. Add 5 ml of barium chloride T.S., digest in a covered beaker on a steam bath for 2 hours, and allow to stand overnight. No precipitate forms.

5. Packaging and storage. Store in well-closed containers.

6. Functional use in foods. Reducing agent; antioxidant.

VI. Description

A. General Characteristics

Stannous chloride is a white, triclinic, crystalline solid.

B. Physical properties

The dihydrate form melts at 37.7°C, decomposing rather than boiling. It is soluble in cold water, 118.7 parts/100 parts of water at

0°C, and infinitely soluble in hot water (081). It is also soluble in tartaric acid, alkali, and 95% ethyl alcohol (081). When dissolved in large amounts of water it forms a basic salt; and if the solution is exposed to the air, a basic stannic salt is formed (119).

C. Stability in containers.

No information available.

VII. Analytical Methods

A. Detection

Sn^{2+} and Sb^{2+}, usually difficult to separate by paper chromatography, have been successfully separated when existing in various ratios, and in dilutions up to M/100. The solvent system formic acid-anisole (1:1) was used for ascending paper chromatographic separation, with the cations being detected with hydrogen sulfide. Total development time was ca. 1 hour, in contrast to other much lengthier chromatographic procedures (113).

B. Quantitation

1. A basic, cross-linked polystyrene type resin in the acetate (De-Acidite FF) was used by Spairs (131) to separate tin successfully from frequent interferences such as phosphate, fluoride, or metals. The stannous ion was oxidized to the stannic form by preliminary treatment with HCl and H_2O_2, this ion then remaining completely adsorbed on the column. It was eluted with NaOH, then acidified with HCl and color developed with phenylfluorone. Over the 4-40 µg range the recovery of tin was 97-108%, and the author indicates the method should be applicable to many biological samples and to stannous fluoride dentrifices.

2. A wet oxidation of organic samples containing tin, which converts all tin to the stannic form, has been recommended by the British Analytical Methods Committee (006). The sample is oxidized with sulfuric and nitric acids, with nitric, perchloric, and sulfuric acids, or with 50% hydrogen peroxide in the presence of sulfuric acid. It is then treated with potassium iodide and extracted into toluene and returned to aqueous solution by extraction of the toluene with sodium hydroxide. The color is developed in an acidified extract with catechol violet. Satisfactory recoveries were obtained in the range of 1-10 µg of added tin, and reproduced in collaborating laboratories when completely colorless digestates were produced. The committee recommends the method for use in the 1-30 µg range.

3. For the 30-150 μg range, the British Committee again recommends wet oxidation of the organic matter, with extraction by dithiozone in carbon tetrachloride, and reaction of an aqueous solution with zinc dithiol in the presence of thioglycolic acid and sodium lauryl sulfate. The red-colored suspension then is measured photometrically. The success of this procedure was also confirmed by collaborating laboratories (007).

4. The dithiol method has been modified by introduction of Marsapon to replace sodium lauryl sulfate as the dispersing agent for the dithiol complex, and the procedure has been accepted as the Polish Standard for determining tin in foodstuffs (036).

5. Wet oxidation and measurement of the orange-colored tin-quercetin complex has been used for the determination of tin in the 10-500 ppm range, that found in canned goods. The authors (067) found recoveries comparable to those obtained with the dithiol method, but the quercetin procedure requires less manipulation.

6. Tin in the 0.2-1.6 μg range has been determined by a modification of the catechol-violet procedure described in Section 2. As the tin-catechol violet complex formed from less than 1 μg cannot be measured spectrophotometrically (under the conditions of that procedure) in the presence of excess reagent, the complex is retained on a column of asbestos that is buffered at pH 3.8. The complex is then eluted by a dilute solution of a surface-active agent and measured spectrophotometrically. The method was specifically developed for application to organotin stabilizers which had migrated from containers into foodstuffs. (In that situation the organotin compound was separated from the inorganic tin by paper chromatography prior to wet oxidation.) However, it should be generally applicable to inorganic tin in that range (002).

7. The iodometric method for the assay of tin content in foodstuffs, a procedure often used in the past and a Polish Standard since 1960, was found to be burdened with a significant negative systematic error. The problem was studied and discussed in some detail by Fitak and Rajpert (029), and a modified procedure recommended. The later method calls for a lower pH of the reaction medium and an increase in the amount of reducing agent for reduction of stannic salts to stannous salts. The modified procedure offers a mean recovery of 95.86% and a mean accuracy of a variance 2S=2,9%.

8. Wet oxidation, used in a number of the procedures described above, is believed by other workers to be undesirable. It is time-consuming, requires a number of reagents, is accompanied by literation of toxic vapors and gases, but yet does not totally eliminate metal losses. Also, large quantities of protein and fats can cause noticeable fluctuations in results (091). Dry incineration, on the other hand, offers simplicity, absence of noxious vapors and gases, etc. Hence if the higher metal losses (from evaporation and transition into insoluble states) could be avoided, a more suitable oxidation procedure would result. Such a procedure has been developed by Medvedeva (091), who utilized magnesium acetate to accelerate and to bind the tin in a soluble complex. With this dry incineration procedure determinations were accomplished with an error of ± 2.38%, and an error not exceeding ± 7.1% in individual instances. This author has also combined his dry incineration procedure with a titration with cacotheline (090). The sample is calcinated in the presence of magnesium acetate, the ash diluted in muriatic acid and titrated against a cacotheline solution (prepared from brucine and nitric acid). The method is sensitive to values as low as 0.005 mg of tin, with an error not exceeding ± 10%.

9. Another dry incineration procedure (037) has been described by Gajek, and recommended for use as a Polish Standard in preference to wet oxidation. The sample is dried, carbonized over a burner flame, and incinerated in a muffle furnace at 450-500°C. The resulting ash is dissolved in 20 ml HCl (1:1 dilution), diluted further, and the Sn^{4+} reduced to Sn^{2+} by thioglycolic acid, then treated with Mersapon and dithiol (mixed with thioglycolic acid in 1% sodium hydroxide) and the color read photometrically. The procedure has been applied to the determination of Sn in the 5-100 µg range, with an accuracy equal to or better than 5%.

10. Still another dry ashing procedure (045) utilizes a mixture of potassium dihydrogen phosphate and magnesium nitrate. Recoveries were reported as 102.0% for 2 and 5 mg of tin, 99.0% for 10 mg, and 99.7% for 15.0 mg.

11. Tin has been determined simultaneously with lead and copper in fresh fish and various preserved foods by a dry incineration method and spectrographic measurement. An accuracy of ± 5% has been reported by the authors (155) for those samples; and the same group (Zore and Tikhonova)

reported ± 6% accuracy for tin when determined simultaneously with manganese, copper, aluminum, and lead in samples of vegetables and berries (141).

12. Atomic absorption spectrophotometry has been applied in determining tin and iron in fruit juices (112). The method is applicable to concentrations as low as 2.5 ppm, with recoveries of ± 10% and reproducibility with standard deviation of 2.3% (sic).

VIII. Occurrence

The purpose of this monograph is the evaluation of stannous chloride as a food additive. However, since the stannous ion is the pertinent factor in the food additive's action, and since stannous chloride is easily ionized in water solution or acid medium, and easily oxidized, it seems pertinent to report here on the effects of mineral tin compounds wherever they occur. The natural occurrence of tin itself is also discussed briefly. Organotin derivatives are not discussed, as they are significantly different in biological activity.

A. Plants

In southern Vermont, an area known to have a rather high soil tin content, tin in a variety of fresh vegetables ranged from 0.0 to 9.07 μg/g (ppm), but was usually less than 1 ppm (124). Tin content in grains or grain products ranged from 0.0 to 2.48 μg/g. In another report (141) without indication of unusual soil conditions, tin content in vegetables and berries ranged from 0.03 to 0.09 mg/kg (μg/g). Tin is not ubiquitous on the earth's surface (124) and for this reason, as well as for others, is not considered essential to biological function.

B. Animals

In fresh animal tissues, again from an area known to have a high soil tin content, the amount of tin ranged from 0.00 to 7.28 μg/g (124). In fresh fish from the Moscow commercial network (155), tin values from 0.100 to 0.630 mg/kg (μg/g) were noted. These values, as well as those of Section A, were obtained by spectrographic measurement, but the authors (155) indicate that the ranges reported were confirmed by others using nonspectral procedures.

C. Tin in Canned Foods

There are numerous reports on tin in canned goods, and no attempt has been made to survey this area completely. Representative excerpts only are offered to indicate the significance of this source of contamination by the stannous ion. In unlacquered tin cans, Schroeder (124) found tin values of from 3.9 μg/g for chili powder to 129.31 μg/g for anchovies. In lacquered cans he reports much less variation: 2.84 to 11.82 μg/g in seven samples of vegetables and fruit.

In a study of storage effect variation with temperature (017), fruits in plain tinplate cans contained 22-48 mg/kg (ppm) when stored at 1°C, and 254-538 mg/kg at 37°C. Tin content in meat products in the same type of can varied from 15 to 48 mg/kg at 1°C and from 43 to 302 mg/kg at 37°C. In enameled tinplate cans, meat products contained no traces at 1°C and 8 and 12 mg/kg at 37°C in 2/6 samples tested.

In evaporated milk samples (001) held under refrigeration for up to 4 years there was no significant change in tin content with time (compared to fresh controls) but considerable variation from can to can. Tin content for duplicate cans from the same milk lot showed, for example, variation from 5.4 ppm to 1.4 ppm. The degree of etching of the tin plate was interpreted as a causative factor.

Little attention has been given to the tin uptake in fatty and fat-rich foods in general, even though unusually high tin values have been reported by several authors for anchovies (011,138). They found the uptake in fat-rich foods was generally less than 1 mg/kg, with occasionally considerable variation between individual cans (011). Higher values were noted in cans that were dented, and particularly in oval flat cans with a roll-up lid where the contact with solder is apparently particularly pronounced. In lacquered grooved cans, low values (25-35 μg/kg) were noted, while in lacquered overlap-soldered cans, a somewhat higher uptake was noted (40-50 μg/kg).

D. Other Natural Sources

In sea water, tin is present in amounts of about 3ppb; none was found in fresh water (28 major rivers at 71 locations), but less agreement on municipal waters was found, with reports varying from 0.8-30 ppb. In air, 0.003-0.3 μg/m^3 were found in 60% of the samples from 22 cities (124).

BIOLOGICAL DATA

I. Acute Toxicity

Table 1. Acute Toxicity of the Stannous Ion

Substance	Animal	Sex and Number	Route	Dosage mg/kg Body wt	Measurement	Reference
Stannous chloride	Mouse	-	p.o.	215	LD_{50}	111
Stannous chloride	Dog	-	p.o.	200	LD	025
Stannous chloride	Dog	-	i.v.	20-50	LD_{100}	130
Stannous potassium tartrate	Mouse	-	s.c.	70(Sn)	LD	048
Stannous potassium tartrate	Guinea pig	-	s.c.	70(Sn)	LD	048
Stannous potassium tartrate	Rabbit	-	s.c.	70(Sn)	LD	048

In table 1 dogs with a reported oral LD of 200 mg/kg of stannous chloride (025) were observed to have grave and fatal intestinal disorders, and this same dose intramuscularly (i.m.) caused convulsions, dyspnea, and fatal paralysis. In the animals administered stannous potassium tartrate (048), signs of paralysis developed within a short time, starting with the hind legs, then rising until the animal died in an asphyxial seizure.

Humans

An outbreak of gastrointestinal distress among a party of women was reported by Warburton et al. (151). Thirty-eight women were served a buffet, and by the following morning 31/37 reported illness. The onset was abrupt, with bloating, then overwelming nausea, stomach cramps, and vomiting;

and diarrhea in one-third of the patients. A few had blurred vision, fever, fainting, and sore throat, and one reported a rash. No analyses of biological samples from the patients were attempted, but analyses of all the foods served were made. No bacterial contamination was found, but the vodka fruit punch was found to contain 2000 mg of tin per liter of punch. No evidence of copper, zinc, or cadmium was found. The punch, made from reconstituted pineapple-grapefruit juice, had a pH of ca. 3, indicating high acid content. It was delivered to the restaurant in a retinned 5-gallon milk can, which on examination after the incident, showed obvious evidence of corrosion, and scrapings from the bottom of the can contained large amounts of tin.

II. Short-Term Studies

A. Mice

1. Toxic doses, by repeated injection, have been reported by Deschiens et al. (205). Eight mice were given stannous chloride, i.m., 60 mg/kg/day for 6 days. This total of 360 mg proved to be lethal. The anthelmintic medical dose for S. mansoni as reported by these authors (4 mg/kg/day for 8 days) is apparently well tolerated. However, they stated that the intoxicated animals showed hepatic and renal changes (parenchymatous hepatitis and epithelial nephritis), and on autopsy the liver showed dark brown granular deposits that were shown by spectrographic examination to be rich in tin. These liver deposits were also found in the animals receiving the medicinal doses.

B. Guinea Pigs

Deschiens et al. (025) have also recorded a total toxic dose of 240 mg/kg, administered as 40 mg/kg/day i.m. for 6 days, for these animals. The medical dose, 4 mg/kg/day for 6 days, again was apparently well tolerated. In the animals of the higher dose group, there were lesions of the lungs, revealing a congestion of small hemorrhagic foci, a parenchymatous hepatitis, and an epithelial nephritis. Before death, dyspnea, watering of the eyes, a generalized trembling, and paralysis of the rear quarters were noted. Spectrographic examinations of the liver and kidney tissues were positive for tin.

C. Rabbits

1. A total dose of 240 mg/kg/day, i.m., the same as for the guinea pigs, resulted in the same clinical symptoms and pathological lesions (025).

2. Stannous potassium tartrate was administered to three female rabbits (048). One was given a total of 46 mg Sn/kg subcutaneously (s.c.) in 4 days, this proving to be lethal. The second was given a total of 18.0 mg Sn/kg s.c. in 8 days, the animal expired in 8 days. The third received a total of 813 mg Sn/kg over a period of 2 months, and died on the 51st day. Other rabbits were administered an organic tin complex, a pyrocatechol derivative. It proved to be of lower toxicity in both acute and chronic situations, but as pointed out earlier, the organotin compounds do not behave chemically in a manner sufficiently similar to stannous chloride to warrant comparing results.

III. Long-Term Studies

A. Mice

Schroeder (125) has reported a long-term feeding study on random-bred Charles River CD mice. Fifty-four males and 54 females were given basic water to which 5 μg/ml Sn as stannous chloride had been added. The water also contained 62.5 μg/ml ascorbic acid to inhibit oxidation to stannic tin. In addition, their diet contained tin 0.28 μg/g (wet weight). Growth rate was unaffected, and the tin did not appear to affect mortality at any age. In general, life-span and longevity (mean age of the last surviving 10%) did not appear affected when compared with controls. There was accumulation of tin in a number of the organs (to be reported in a later section), but the author concluded that in spite of this, innate toxicity was not demonstrated. The one effect noted was that the median life-span of males and females was almost identical and the longevity of the males greater than the females. Usually females live longer than males, according to other studies this author quotes.

B. Rats

Life studies on Long-Evans rats were also reported by Schroeder (126). Stannous chloride was again administered in the drinking water, 5 μg/ml, to males and females, with more than 50 in each group. Growth, serum glucose, and cholesterol levels were followed and, at natural death,

weights and gross and microscopic pathology were studied. Males exhibited one adverse effect, lower body weight, while females exhibited decreased survival and longevity, and elevated glucose levels. Both males and females had increased incidences of fatty changes in liver and renal tubular vacuolization. These changes were found in the presence of only small amounts of tin accumulation in the tissues. Also, it should be noted that no criterion was altered in a favorable direction.

IV. Special Studies

A. Teratology

1. Stannous chloride has been evaluated for teratological effects on mice (031). Four groups of virgin adult female albino CD-1 outbred mice (26, 25, 22, and 25) were mated with young adult males, and beginning on day 6 of gestation were treated daily through day 15, by oral intubation, with 0.5, 2.3, 11.0, or 50.0 mg/kg stannous chloride in water solution. Controls were sham treated, or given 150 mg/kg aspirin as positive control. On day 17 all females were subjected to Caesarean section and the numbers of implantation sites, resorption sites, and live and dead fetuses recorded. Body weights of live fetuses were recorded, and the urogenital tract of each mother was examined. All fetuses were examined grossly for the presence of external abnormalities. After sacrifice one-third of the young of each litter underwent detailed visceral examination and the remaining two-thirds were cleared in potassium hydroxide, stained with alizarin red S dye, and examined for skeletal defects. The number of abnormalities did not differ from the spontaneously occurring ones in the sham-treated controls, and the authors concluded that: "The administration of up to 50 mg/kg of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

2. Albino Wistar rats and golden hamsters were treated similarly (031), given the exact dosages as those described, with the same negative conclusions regarding nidation, survival, and abnormalities.

3. A human case of right-hand agenesis has been reported (106)

that is attributed to an antiparasitic drug containing tin taken by the mother at the end of the second month of pregnancy. To eliminate the existance of a Taenia saginata in her body, the mother was given 3 tablets per day, for 5 days, of a taenifuge of 60 mg of pure tin oxide (the stannous or stannic form not specified) plus 60 mg pure metallic tin per tablet. These tablets were given in conjunction with 250 mg of pyridoxine per day, on the first three days of the 5-day treatment period. The authors believe the metallic substances were not absorbed by the body but were rejected with the feces after having acted on the parasite in the intestinal environment. They believe the inorganic forms behave differently than organic derivatives of tin (for example, iodized or ethylic derivatives), which are perfectly absorbed and known to be teratogenic. They postulate that, rather than absorption with crossing of the placental barrier, the effect was due to an association with pyridoxine, which intervenes in numerous metabolic transformations, specifically as a transamination and decarboxylation agent. However, others (025) indicate that stannous oxide is at least partially transformed by hydrochloric acid in the stomach to stannous chloride, an ionized form which is absorbed and passes to other parts of the body. This absorption seems to have now been firmly established, as will be noted by numerous other references in this monograph.

B. Embyronic Studies

The effects of metals on the chick embryo have been reported by Ridgway (116) who gave data on 54 elements in various forms. Stannous chloride was injected by two routes, giving an LD_{50} of 10.0 mg/egg on a 4-day embryo when administered by yolk sac route, and an LD_{50} of 7.40 microatoms when given to an 8-day embryo via the chorioallantoic membrane. A microatom was defined as:

$$\left(\frac{\text{mg/egg} \times 1000}{\text{mol. weight of the salt in g}}\right) \times \text{(no. of atoms of element in the salt)}$$

No teratogenic effect was noted for stannous or stannic chloride, by that experimental procedure (116).

C. Dermal Inflammation

The effects of stannous chloride and stannous fluoride on inflammation have been reported by Stone and Willis (134). Both chemicals will be mentioned briefly here, from the standpoint of the stannous ion, as already discussed. Sixteen white rabbits were clipped over the abdomen 2 days before testing. They were scratched deeply enough to break through the epidermis, but with little or no bleeding. Distilled water suspensions of 2, 1, 0.5, and 0.25% stannous chloride, 0.3 ml on a piece of gauze, were applied to a scratch, with two control sites of distilled water. Stannous fluoride was applied similarly at 0.5, 0.25, 0.1, and 0.05% concentrations with two control water sites. The test sites were then protected by gauze covering, taped, and the patches removed and read after 18 hours. A second group of 10 rabbits was not scratched, 5 receiving 1% stannous chloride at two sites with two water control sites, and 5 receiving 0.25% stannous fluoride in the same manner. The results were again read at 18 hours.

Of the first group (scratched), the control sites consistently had no pustules. Test sites of 2% and 1% stannous chloride always had a continuous row of pustules on each side of the scratch. The pustules never occurred at the lower concentrations. Biopsy showed a marked polymorphonuclear leukocyte reaction that destroyed the epidermis and the upper dermis. Sites of lower concentration than the 1% stannous chloride and 0.25% stannous fluoride did not produce pustules but still produced a polymorphonuclear infiltrate that was markedly increased over controls. Control scratch sites had very little inflammation. In the unscratched test sites (group 2) no pustules were noted in control and test sites, gross or by biopsy.

It seems pertinent to point out, however, that due to the basic nature of the stannous chloride solution (see Section VI, Chemical Information) this local inflammation may be partially due to pH.

D. Stannous Salts as Anticaries Agents

There are numerous reports in the literature on the use of stannous salts as anticaries agents, but for the purposes of this monograph brief mention of a few of the studies should suffice. Muhler (098)

studied the effect of vanadium pentoxide, fluorides, and tin compounds on the dental experience in rats. Sprague-Dawley strain rats were divided into 7 groups according to sex and initial body weight and given supplements of tin and fluoride compounds in drinking water administered for 840 days.

On the basis of these results (see Tables 2 and 3) the author concluded that caries rates were significantly reduced by stannous fluoride, potassium fluorostannite, and by stannous chloride and stannous gluconate to which sodium fluoride had been added. The stannous salts alone (without fluoride) were essentially without effect. Francis (034) generally confirmed these results, finding that stannous chloride applied topically at 2% was ineffective. Stannous fluoride supplied at 10 ppm in drinking water was markedly superior to sodium fluoride at the same fluoride concentration, while stannous chloride at an Sn^{2+} level similar to that of stannous fluoride was ineffective. Gray (047) studied the dissolution rates of sound and white-spot enamel on human teeth when treated with tin(II) and fluoride compounds. The teeth were treated *in vitro* with stannous fluoride, stannous chloride, or sodium fluoride at 3000 ppm Sn^{2+} and 950 ppm F^-. The dissolution rate was measured in lactate buffer by both differential and integral methods. Treatment with stannous fluoride reduced the dissolution rate of either sound or white-spot enamel, and the effect was always equal to or greater than that effected by treatment with stannous chloride or sodium fluoride under comparable conditions.

E. Carcinogenicity

Mice and Rats

1. As a part of the long-term study discussed earlier (125), Schroeder studied the effects of arsenic, germanium, tin, and vanadium on spontaneous tumors in mice (062). Eight-six random-bred white Swiss mice of the Charles River strain (CD) were exposed from weaning until death to doubly deionized drinking water containing the essential trace metals manganese (10 μg/ml), chromium (1 μg/ml), cobalt (1 μg/ml), copper (5 μg/ml), zinc (50 μg/ml), and molybdenum (1 μg/ml) as the acetate, citrate, or molybdate. To this had also been added 5 μg/ml stannous chloride (stabilized with ascorbic acid). Although it was

Table 2 (098)

Comparison Between the Dental Caries Experience of Rats Receiving Different Tin Compounds in Their Drinking Water (Series I)

Group No.	Supplement	Fluorine conc. (µg/ml)	Tin conc. (µg/ml)	No. of rats	Mean No. of lesions	Caries reductions[a] (%)
1	Stannous gluconate[b]	--	90	25	7.2	8
2	Stannous gluconate plus fluorine[c]	30	90	26	4.5	12
3	Stannous chloride	--	90	29	6.9	12
4	Stannous chloride plus fluorine[c]	30	90	33	4.9	37
5	Stannous fluoride[d]	30	90	30	3.7	53
6	Stannous chloride	--	180	19	6.4	18
7	None	--	--	33	7.8	--

[a]Based upon number of carious lesions.
[b]Furnished by the Metal and Thermit Research Laboratory, Rahway, N. J.
[c]As sodium fluoride.
[d]Prepared at Indiana University by Mr. Joseph Davis to whom we are indebted. See J. Am. Chem. Soc. 71: 1604, 1952, for method of preparation.

Table 3 (098)

Comparison Between the Dental Caries Experience of Rats Receiving Different Fluoride Compounds in Their Drinking Water (Series II)

Group No.	Supplement	Fluorine conc. (μg/ml)	Tin conc. (μg/ml)	No. of rats	Mean No. of lesions	Caries reductions[a] (%)
1	Potassium stannous fluoride[b] (potassium fluorostannite)	30	60	26	5.6	32
2	Stannous fluoride[c]	30	90	29	5.0	41
3	Stannous fluoride plus sodium fluoride	30	45	31	7.2	15
4	Stannous fluoride plus potassium fluoride	30	45	28	6.8	19
5	None			30	8.4	

[a]Based upon number of carious lesions.
[b]Furnished by Dr. William F. Bruce, 900 North Broad Street, Philadelphia, Pa. to whom we are indebted.
[c]Prepared at Indiana University by Mr. Gino Bisagio to whom we are indebted.

difficult to measure, the author estimated an intake of 35 µg/100 g/day. The diet also contained 0.28 µg/g tin. The group was followed for 33 months, by which time a total of 64/86 autopsies had been performed. Twenty-two tumors were found, including 6 malignancies, with 25.6% of the animals tumor-bearing. Controls in the same period were found to have 32.4% of the group tumor-bearing. The types of tumors found also corresponded to those of the controls, with the ratio of malignanat to benign essentially the same. From these data the author concluded that, in the dose given, stannous chloride was not carcinogenic.

2. Similar studies were carried out on rats by Schroeder (126). The drinking water contained 5 µg/ml stannous chloride, plus the essential trace metals listed in the preceding section. In the controls 37.8% of the rats had spontaneous tumors, while in the stannous chloride-treated group 29.9% were found to be tumor-bearing.

3. It is interesting to note that studies by Roe et al. (150) have shown that Chester Beatty stock mice receiving 1000 or 5000 ppm tin as sodium chlorostannate (tetravalent tin) in drinking water or 5000 ppm tin as stannous oleate in the diet experienced lower incidence of malignant lymphoma, hepatoma, and pulmonary adenoma than the untreated controls.

In 30 inbred August hooded rats fed a diet containing 2% chlorostannate (117) for a year, one mammary adenocarcinoma, one uterine pleomorphic sarcoma, and one jaw adenocarcinoma were found. In 27 rats on a diet containing at first 1% then 0.5% stannous 2-ethyl hexoate, no neoplasms were found. In 33 untreated controls followed for the same period, no malignancies were found. The authors consider the results promising, but believe a larger experiment is needed to establish non-carcinogenicity with certainty.

BIOCHEMICAL ASPECTS

I. Breakdown

Stannous chloride, when present in foods, particularly in an acid medium, undoubtedly undergoes ionization, with the Sn^{2+} ion entering into complexes or equilibrium situations with other ions present. For example, Van Buren (145) in studying the effect of tin on waxed beans observed that the best color was found in beans canned in unlined tin cans and a poorer color in enamelled cans. In studying the reaction he found that the dark color, induced by the addition of iron, which is postulated as forming a combination with tannins, can be reversed by addition of stannous chloride. He proposed the equilibrium:

$$3\ Sn^{2+} + 2\ Fe^{3+} \rightleftharpoons 3\ Sn^{4+} + 2Fe$$

Also, Sn^{2+} can replace Fe^{3+} in the polyphenol complex, as was observed by Hernandez (050) in his work on asparagus. Indeed, the ionization of stannous chloride to free the Sn^{2+} seems to be the basis of its usefulness as a food additive, i.e., producing a "cosmetic effect." The stannous ion has been shown to be the active agent in inhibiting the pink discoloration in pear puree (019), perhaps through reaction with the products of the breakdown of leukocyanidin at the quinone methine stage. In an attempt to prevent darkening of glass-packed sauerkraut (040), it was observed that stannous chloride at the 5 ppm level showed promise in retarding darkening, but in samples containing 5-15 ppm color was not uniform, and a precipitate formed which turned some shreds black, giving an effect of light and dark layers. It seems likely that a chemical reaction between the stannous ion and a constituent of the sauerkraut had taken place. The sauerkraut was maintained at an acid level of 1.0% as lactic acid.

In beer (092) the cloudiness which appears at times in some beers in varying degrees has been attributed to the stannous ion and its affinity for certain groupings of proteic molecules, either through adsorption or combination.

II. Absorption and Distribution

A. Mice

Long-term studies by Schroeder (125) already discussed from the standpoint of growth, longevity, etc. (Biological Data, Section III), included tissue levels of tin. The Charles River CD strain males and females, after receiving 5 μg/ml of tin as stannous chloride ad lib in their drinking water for their life-span, accumulated tin as shown in Table 4.

At these levels, as already pointed out, the author concluded that toxicity was not demonstrable.

B. Rats

1. A similar pattern of accumulation was found in rat tissues (126) as shown in Table 5. It is interesting to note that in 9 of the analyses on 23 stillborn or suckling rats no detectable tin was found in their whole carcasses. Three contained 0.68 μg/g. Certain tumors accumulated tin in concentrations of 0.91 to 7.77 μg/g, 5 containing more than 2.73 μg/g, while 4 other tumors had no detectable tin. Hearts and spleens showed more tin than did other normal tissues, the same as with mice.

2. In therapeutic doses (numerical values not specified) of stannous chloride or that form of Sn associated with tin oxide (142), tin was found to have accumulated in the digestive tract, liver, vessels, spleen, kidneys, lungs, skin, and exoskeletons, and the brain of rats. In this last organ tin was found not only in the pia mater and intracerebral vessels but also combined with proteins and with lipids in the interior of the pyramidal cells. These results were obtained from oral dosage, and also from s.c., intraperitoneal (i.p.), or intravenous (i.v.) administration, although in the case of skin, nails, hairs, and teeth, the results were on the basis of oral or s.c. treatment. In the brain, parenteral administration resulted in less localization than from the oral route.

C. Rabbits

Therapeutic doses administered to rabbits (142) were accumulated in the same general areas as with rats, as described above.

Table 4 (125)

Tin in Mouse Tissues, Wet Weight[a,b]

	Males		Females	
	No. animals	µg/g	No. animals	µg/g
Kidney	29	1.70	37	3.32
Liver	29	1.24	37	2.26
Heart	43	2.95	32	5.04
Lung	32	4.47	37	1.80
Spleen	43	1.81	37	4.47
Fat	--	--	2	0.81
Thyroid	14	2.15	--	--
Tumor	--	--	1	5.12

[a]Organs were pooled in lots of 3-14. Food contained 0.28 µg/g tin. Mean values shown.
[b]Too few control tissues were avilable for tabulation; they contained less than 0.5 µg/g tin.

Table 5 (126)

Tin in Tissues of Rats, Wet Weight

	Controls[a]			Fed tin[b]		
	No. rats	No. present	Mean µg/g	No. rats	No. present	Mean µg/g
Kidney	21	7[c]	0.31	32	32	0.17
Liver	21	16[d]	0.11	32	32	0.35
Heart	21	18	0.59	32	32	0.93
Lung	18	7[c]	0.14	32	32	0.54
Spleen	21	21	0.96	36	36	1.88
Stillborn, whole	26	3	0.08	—	—	—
Tumors	18	11	1.77	2	2	1.12

[a]Control rats were 523 to 822 days old.
[b]Tin-fed rats were 602 to 953 days old.
Tissues were pooled in groups of 3 to 11 samples.
Differences between the means of the control and rats fed tin were not statistically significant.
[c]Differs from group fed tin by chi-square analysis, $P < 0.001$.
[d]$P < 0.01$.

D. Man

1. Tin has been found in 29 organs and tissues of American adults (124). The highest concentrations were found in the intestinal tract where 92% of 637 samples showed it. (On human tissue tin was analyzed by the spectrographic procedure of Tipton et al., Health Physics 9: 89 (1963)). Median concentrations altered caudally as follows (ppm of ash): esophagus 18, stomach 16, duodenum 28, jejunum 36, ileum 79, cecum 130, sigmoid colon 45, and rectum 57. The author points out that this pattern appears related to reabsorption of fluids from intestinal contents. Sizable amounts were found in other tissues: lung 37, adrenal 23, and liver 23 ppm. Only 5.4% of samples indicated tin in the brain (as opposed to the results from therapeutic doses on rats and rabbits) and 19% in muscle; and diaphragm, heart, and pancreas had low concentrations. In no single tissue was tin always found, although all subjects showed it in many organs. Bone accumulated variable to large amounts. The author stressed that there were variations in tissue, and occasionally adult cases had very high or very low concentrations. In aorta, kidney, and liver there were three samples with levels greater than 150 ppm ash (1.65 ppm wet weight) and in lung 2/9 samples contained 880 ppm. Five samples from the gastrointestinal tract contained more than 1000 ppm. In contrast, in 100-145 samples there were no traceable amounts of tin in 5 lungs, 7 livers, 9 aortas, 23 spleens, 66 pancreases, and 17 kidneys. Even 20/438 samples of intestinal tract were without tin, as were 30/199 samples of esophagus and stomach. Variations with age are shown in Table 6. The author interprets the wide variation in infants and children as an indication that tin does not cross the placental barrier.

Geographical variations are illustrated in Table 7.

2. In an earlier study on trace metals, the concentration of tin in normal females, not employed or associated with industry, was reported (063). Table 8 illustrates the results of this survey.

III. Metabolism and Excretion

A. Rats

Comparative studies on the reabsorption of heavy metals *in vivo* and *in vitro* have been offered by Leopold et al. (083). Utilizing previous

Table 6. (124)

Tin in Human Tissues by Age, Mean U.S. Values (ppm ash)

Age group (yr)	0	0-1	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-84
Kidney										
Concentration	0	57[a]	60[a]	33	20	34[a]	28[a]	22	34	32
Occurrence	0/16	9/10	19/20	10/15	17/21	36/43	22/26	26/27	13/14	8/9
Liver										
Concentration	0	48[a]	61[a]	42	34	33[a]	25	35	38[a]	34[a]
Occurrence	0/21	11/12	22/23	14/14	20/21	39/43	27/28	25/25	13/13	9/9
Lung										
Concentration	(18)	35	34	45[a]	27	31[a]	39[a]	53[a]	58	64[a]
Occurrence	1/10	5/6	5/6	10/13	18/21	34/35	27/27	28/29	13/13	9/9
Ileum										
Concentration	0	101	98	80	174	116	97	53	172	140
Occurrence	0/8	1/2	1/1	9/10	12/13	26/28	16/17	12/12	6/6	3/4
Total occurrence (%)	1.8	86.7	94.0	82.7	88.2	79.8	93.9	97.8	97.7	93.5

Note: Concentrations of positive samples.
Occurrence refers to the number positive/total cases.
[a]Excluding vales >150.

Table 7 (124)

Geographical Distribution of Tin in Kidney, Liver, and Lung of Man (ppm ash)

	No. of samples	% present	Mean	Range	Median
Kidney					
United States	161	97	30	<5-480	20
Switzerland	9	33	5	<5- 17	<5
Africa	53	19	5	<5- 30	<5
Middle East	43	42	8	<5- 55	<5
Far East	57	40	11	<5-110	<5
Liver					
United States	163	96	35	<5-300	24
Switzerland	9	78	6	<5- 20	Tc
Africa	49	25	4	<5- 20	<5
Middle East	44	55	11	<5-140	Tc
Far East	54	41	10	<5- 74	<5
Lung					
United States	159	98	69	<5-920	39
Switzerland	7	100	37	23- 62	28
Africa	50	60	10	<5- 40	Tc
Middle East	45	91	33	<5-200	17
Far East	57	93	65	<5-1200	29

Note: Tc = trace, or barely detectable.
Mean and median values were obtained by assigning a value of one-half the least detectable amount to those cases where tin was not detected.

Table 8 (063)

Concentration Levels of Trace Metals in Normal Human Tissues (American)
(arithmetical mean values)

Tissue	Milligrams of Metal per 100 g wet tissue					
	Mn	Pb	Sn	Al	Cu	Ag
Kidney	0.060	0.027	0.020	0.042	0.166	0.00
Heart	0.032	0.038	0.022	0.056	0.190	0.00
Brain	0.030	0.013	0.00	0.004	0.400	0.003
Liver	0.205	0.130	0.060	0.160	0.710	0.005
Spleen	0.022	0.030	0.022	0.130	0.085	0.00
Lung	0.022	0.028	0.045	5.94	0.110	0.004
Muscle	0.050[a]	0.010	0.011	0.015	0.125	0.00
Long bone	0.300[a]	1.88	0.080	0.500	1.190	0.00
Rib bone	0.170[a]	0.470	0.050	0.240	0.410	0.01
Stomach	0.030[a]	0.022	0.050	0.073	0.107	0.00
Intestines	0.035	0.023	0.016	0.087	0.110	0.002

[a]Results of single analyses.

knowledge regarding the transport system for iron which floats across the mucosa localized chiefly in the jejunum, a comparison of the penetration and binding of heavy metals in the ileum and jejunum was made. Measurements were made in vitro on small everted intestinal sacs and on tied intestinal loops on the rat in situ. The authors concluded on the basis of their studies that tin or the other heavy metals were not utilized in this floating-out mechanism.

B. Rats and Rabbits

The fate of organic and inorganic derivatives of tin administered parenterally and orally has been studied by histochemical means (066). Dosages, strains, and specific experimental conditions for the tests on rats and rabbits were not given, but the experiments were generally described as "acute intoxication," "massive," "subacute," and "chronic," and will be referenced in that way in discussion. In acute intoxication the compounds administered orally and parenterally were recovered in the liver, in the blood vessels, and in the elements of the blood, but very little in the hepatic parenchyma. The tin congests the liver at the level of the vessels and capillaries, which are dilated, and the Kupffer cells "fix or set" the tin. The organic derivatives cause only a few lesions in the hepatic parenchyma (death was more rapid from nervous intoxication), while the mineral derivatives (stannous chloride, Stanoxyl, or tin oxide in association with tin chloride) were more irritating to the vascular walls. In subacute and chronic intoxications, which approximate therapeutic conditions, and in accidental intoxication, the tin compounds are stored in larger quantities in the hepatic cells. It was found that the tin stored in the liver was eliminated by the blood and kidneys rather than by the biliary route, and also it was observed that there were differences and individual reactions in relation to the tin salts, organic or mineral.

C. Man

1. In an early study on the excretion of tin by man (064) two normal men were given a total of 28 mg of Sn as Stanoxyl by daily i.v. injection during the second week of a 21-day metabolism experiment. The first week served as a control period during which the absorption and

excretion of the Sn present in the food was followed. The results are shown in Table 9. Both men were roughly in balance during the first period, as the author points out, excreting 52 to 75% of the whole output of Sn in the urine. During subsequent periods the injected Sn was excreted slowly. The figures presented by this author are much higher than those of others (see 017, 063, and 124). Perhaps this is due to differences in analytical measurement or other dissimilar parameters.

2. The urine of a healthy experimental man was examined for 28 consecutive days (063) with the results shown in Table 10. The subject's food, beverage, and feces metal content were measured during the same period, with the results shown in Table 11. From these data the author concluded that the daily output in the feces is practically equivalent to the daily intake in the diet.

3. The fecal excretion of iron and tin by men fed on a diet of stored canned foods has been reported (017). Table 12 indicates the metal content of the various rations which had been stored at 1° or 37° C for 20 months. Fecal metal excretion values are shown in Table 13. Of the tin ingested from the rations stored at 1° C, practically all was excreted by the feces, confirming the results of Kehoe (063) and others.

IV. Effects on Biochemical or Morphological Parameters

A. Mice

In the study of the acute toxic effect of stannous chloride on white mice (111), a dose of 1000 mg/kg (ca. 5 times the LD_{50} reported by the same authors) was administered in lemon juice sweetened with sugar. Dosage was by stomach tube to 3 males and 3 females, with sacrifice 8 hours after administration. The experiment was repeated three times on the same number of animals. Gross examination showed expanded intestines, serosa considerably congested, single petechiae on the stomach serosa, spastic and dilated sections alternating along the small intestine, livers darker red with tissue homogeneous, and dark red hyperemic lungs. Histologically the liver showed necrosis, with disintegrating leukocytes in some; in other parts of the liver parenchyma the cells had parencymatous cloudy swelling or showed vacuolar degeneration, and in some fatty degeneration. There was also necrosis of the spleen.

Table 9 (064)

The Absorption and Excretion of Tin

Subject	Length of period days	Sn intake mg/period Injected during 2nd week	Sn intake mg/period In food	Sn output mg/period In urine	Sn output mg/period In feces mg	Balance period	Sn recovered mg
E. B.	7	0	14.4	7.2	6.6	0.6	
	7	28	13.1	12.6	7.3	21.2	19.7
	7	0	12.4	13.4	11.9	12.9	
R. M.	7	0	12.8	8.8	2.9	1.1	
	14	28	30.5	36.6	10.6	11.3	16.7

Table 10 (063)

Concentrations of Trace Metals in Successive Daily Urine Samples of a Normal Adult American

Milligrams per liter	Frequencies of occurrence of the quantities indicated					
	Mn	Pb	Sn	Al	Cu	Ag
0.00 –0.009	28		7	1	2	28
0.010–0.019		1	17	1	5	
0.020–0.029		8	3	3	7	
0.030–0.039		14	1	5	3	
0.040–0.049		4		4	5	
0.050–0.059		1		4	1	
0.060–0.069				5	2	
0.070–0.079				1	2	
0.080–0.089				1		
0.090–0.099				2		
0.100–0.109						
0.110–0.119				1	1	
Number of samples	28	28	28	28	28	28
Mean	0.01	0.034	0.014	0.052	0.037	
Probable error	± ?	±0.001	±0.001	±0.003	±0.002	
Standard deviation	± ?	±0.008	±0.007	±0.025	±0.014	

Table 11 (063)

Comparison of the Mean Daily Quantities of Trace Metals in Successive Samples of the Feces and Food of a Normal Adult American

	Milligrams per 24-hour sample					
	Mn	Pb	Sn	Al	Cu	Ag
Food						
Mean	4.28	0.290	17.14	36.43	2.32	0.088
Probable error	±0.43	±0.027	± 1.30	± 7.90	±0.28	±0.010
Standard deviation	±3.38	±0.213	±10.17	±61.97	±2.21	±0.077
Feces						
Mean	3.69	0.320	22.88	41.92	1.96	0.058
Probable error	±0.30	±0.019	± 1.36	± 6.19	±0.18	±0.005
Standard deviation	±2.30	±0.141	±10.28	±46.80	±1.33	±0.035

Table 12 (017)

Tin and Iron Content[a] of C Ration Components Stored for 20 Months at the Indicated Temperatures

Component	Menu No.	Iron 1°C	Iron 37°C	Tin 1°C	Tin 37°C
Packed in 211 by 304 cans 8 oz net weight					
Plain tinplate					
Apricots	6	3	5	32	414
Cherries, white	4	6	52	48	470
Fruit cocktail	3	3	10	43	427
Peaches	1,5	4	8	26	254
Pears	2	5	102	22	538
Packed in 300 by 308 cans 11.5 to 12 oz net weight					
Plain tinplate					
Beans w/franks	1,3	18	22	48	262
Beans w/pork	2	17	15	30	144
Beef stew	4	15	15	22	94
Chop suey	5	15	18	27	169
Hamburger	5,6	34	34	15	43
Meat w/beans	4	24	19	46	302
Meat w/spaghetti	1	15	20	38	288
Enameled tinplate					
Beef w/peas	6	28	130	NF	NF
Chicken w/noodles	1,6	11	14	NF	Trace
Chicken w/vegetables	2,3	24	17	NF	12
Ham and potatoes	3	10	40	NF	8
Meat w/noodles	5	15	22	NF	NF
Pork sausage patties	2,4	24	71	NF	NF

[a]mg per kg of wet food (ppm)
NF = none found.

Table 13 (017)

Fecal Iron and Tin Content

Subject	Order of feeding[a]			Iron (mg/day)			Tin (mg/day)		
	A	CI	CII	A	CI	CII	A	CI	CII
A	1	2	3	16.6	20.7	57.5	10.8	27.0	190.7
B	2	3	1	17.5	22.2	13.4	9.7	30.0	137.0
C	3	1	2	19.7	21.0	39.3	11.9	34.8	150.1
D	1	3	2	17.1	22.1	29.6	12.3	31.1	148.5
E	2	3	1	18.5	20.3	19.7	9.4	29.8	164.9
F	3	2	1	18.4	23.8	35.4	10.9	33.8	109.3
G	1	2	3	18.2	20.5	48.2	9.9	27.2	180.8
H	2	1	3	19.8	20.7	38.3	12.0	32.1	153.1
I	3	1	2	15.9	18.6	33.1	9.6	35.5	169.6
Average fecal excretion....				18.0	21.1	34.9	10.7	31.3	156.0
Average dietary intake.....				22.1	26.7	64.4	9.5	26.3	162.8

[a]Order in which diets A (fresh control), CI (ration stored at 1°C) and CII (ration stored at 37°C) were administered for 24-day feeding periods.

B. Rats

1. Motor end plates in the tibialis anterior muscle were studied in regard to the binding by divalent metal ions (102). Five percent stannous chloride in 0.03 N KCl was utilized. Rats were anesthetized and the muscle exposed; the metal ion was then injected as 0.3 ml/100 g body weight. The muscle was excised 30 minutes after injection and frozen sections made. It was found that stannous chloride behaved in almost all cases as did lead acetate. It was shown that the metal binding activity is a distinct and separate action from cholinesterase activity. The results also suggested that the site of binding of the divalent ions is in close relationship to the site of calcium release and in close but not identical relationship to the site of the cholinesterase activity and the acetylcholine receptor.

2. The effect of metallic salts on the histology and functioning of the rat testis and epididymis has been studied by Hoey (052). Stannous chloride was administered, s.c., 0.04 mmoles/kg, to 5 groups of male albino rats on various schedules. Acute changes were manifest by shrinkage of the tubules, particularly the more centrally placed ones, to give wide spaces between the tubules in which the isolated interstitial tissue is found. Eosinophilic edema fluid was always present in the spaces, and there was a great increase in the lymphatic drainage, all lymph vessels being grossly enlarged, some with breakdown of hemoglobin. Similarly, there was marked angorgement of the intertubular capillaries and testicular veins. Spermatogenesis did not appear to be affected. However, the formed spermatozoa frequently showed separation of the head and some tubules had a ring of tailless pycnotic sperm heads around the lumen. The condition of the spermatozoa in the epididymis varied from normal to quite degenerated. The ductuli efferentes showed marked damage, with considerable epithelial proliferation, and areas of complete blockage. Chronic changes were also noted. In most cases the predominant tendency was for the tissues either to recover or to deteriorate, and both processes often occurred in the same testis, some tubules regaining a normal appearance while the neighboring tubules degenerated completely. Some lymphatic drainage resumed normal proportions, but

even with return to normal appearance there was edema fluid still found in the peripheral tubules. Also, recovery of testicular structure does not necessarily mean a return to normal spermatogenesis. Full recovery of the duct systems seldom occurred. In almost all cases the ductuli efferentes showed marked damage, amounting to blockage of the duct lumen, which could lead to very marked dilation of the rete testis and proximal ductuli, and collapse of the distal ductuli. The head and body of the epididymis showed a more marked ability to recover, necrosis being rare. Stannous chloride was compared with silver nitrate, copper sulfate, nickel sulfate, and cobalt chloride. All showed the results described above, each in varying degrees.

C. Rabbits

Tin as a microelement (the levels normally found in food) has been studied in relation to its effect on cholesterol and lecithin levels in blood serum (013). Female rabbits were given stannous chloride at a dosage level of 0.6 mg/kg or 3 mg/kg, injected per os daily for 15 days. Cholesterol and lecithin were measured on the 5th, 10th, and 15th day, cholesterol by the Liebermann-Burchhardt color reaction and lecithin through the phosphorus extracted by the Ostrovsky method (Lab. Work 11: 27, 1961). With both dose groups there was a reduction in cholesterol and lecithin levels in blood serum, illustrated by Tables 14 and 15. From these data the author concludes that the tin participates in the exchange of lipids in the animal organism.

D. Man

A detailed study on the effect of stannous ion on human semen acid phosphatases has been offered by Takenouchi (137). The chemical difference between two choline-phosphoric acid samples which exhibited different behaviors in their hydrolysis by seminal acid phosphatase was investigated. It was found by emission spectral measurement that one of the samples contained a trace of tin, and further study showed that divalent tin exerted a strong inhibitory effect on seminal acid phosphatase, an effect which could, however, be suppressed completely by fructose. The activity of human erythrocyte acid phosphatase was also inhibited by divalent stannous ion, and this inhibitory effect could not be controlled by fructose. It was found that the optimum pH for

Table 14 (013)

Cholesterol Level in Rabbits' Serum upon Injection of Varied Doses of Tin (mg %)

Time after injection of tin	0.6 mg				3 mg			
	n	M	P	%	n	M	P	%
Norm (control)	9	64.4		100	10	57.6		100
5th day	9	40	<0.01	62.1	10	30.1	>0.001	52.1
10th day	9	31.7	<0.01	49.2	10	27.8	>0.001	48.2
15th day	9	42.2	<0.01	65.5	10	51.8	>0.5	89.9

Table 15 (013)

Lecithin Level in Rabbits' Blood Serum upon Injection of Varied Doses of Tin (mg %)

Time after injection of tin	0.6 mg				3 mg			
	n	M	P	%	n	M	P	%
Norm (control)	10	175.5		100	10	151		100
5th day	9	151.6	<0.1	86.3	9	117.2	<0.01	77.6
10th day	10	140	<0.01	79.7	9	107.2	<0.01	70.9
15th day	9	169.4	<0.5	96.5	7	150.7	>0.5	99.8

the hydrolysis of choline phosphoric acid by seminal acid phosphatase is 5.6-5.75.

V. Drug Interaction

Van Dolah and Christenson have studied the chemical inactivation of streptomycin (146). The lowest inhibiting dilutions against Escherichia coli, Bacillus subtilis, and Staphylococcus aureus are shown in Table 16. Along with stannous chloride, both oxidizing and reducing substances were studied, and the authors concluded that the inactivation of streptomycin could not logically be ascribed to an oxidizing or reducing mechanism, but more likely resulted from an interference mechanism or a specific reaction.

VI. Consumer Exposure Information

As discussed in Section VIII on Chemical Information, the consumer can be exposed to tin from a variety of natural and environmental sources. The FDA has amended its earlier limit of the addition of 15 ppm Sn^{2+} (040) to 20 ppm in glass-lined containers with inert tops, for example in asparagus (005), and the NAS/NRC GRAS Survey Report (032) indicates 20 ppm is the usual and maximum use for processed vegetables, with 8.3 ppm as usual and 9.8 ppm as the maximum for processed fruits. In beverages, the usual usage is reported as 11.7 ppm and the maximum as 12.3 ppm. Possible dietary intakes by age group are shown in Table 17 (032). The FAO/WHO Committee on Food Additives (035) states, however, that the interaction of tin and foods should be the subject of further research; their evaluation of available data did not permit assignment of an average daily intake.

The values for the amount of tin added to the nation's food supply, as reported to the NAS/NRC (104), are shown in Table 18. It is interesting to note that in 1963, Schroeder (124) estimated the daily human intake from all sources as about 4 gm, and pointed out that it had decreased in the period 20 years prior to that time. This no doubt could be largely attributed to the improvement in canning methods, and the use of laquered cans.

Table 16 (146)

Agent	Streptomycin hydrochloride	Minimal inhibiting dilution		
		E. coli	*B. subtilis*	*S. aureus*
——	1 mg/ml	250	>500	>500
Stannous chloride 5.0%	——	65	180	110
Stannous chloride 5.0%	1 mg/ml	70	>500	>500

Table 17 (032)

Possible Daily Intakes of NAS Appendix A Substances (Groups I and II), per Food Category and Total Dietary Based on Food Consumption by Total Sample

Substance name (survey no.)	Food category no. name	No. of firms	Age	Possible daily intake, mg Average	High A	High B
Stannous chloride NAS 0213	14 Proced vegs (R)	*	0-5 mo.	.028000	.084000	.028000
			6-11 mo.	.480000	1.120000	.480000
			12-23 mo.	.780000	1.306000	.700000
			2-65+ yr.	1.700000	2.864000	1.700000
Stannous chloride NAS 0213	23 Bev type I (R)	11	0-5 mo.	.028080	.042120	.029520
			6-11 mo.	.265590	.909090	.279210
			12-23 mo.	.634140	1.901250	.666660
			2-65+ yr.	1.216800	3.249090	1.279200
Stannous chloride NAS 0213	All categories	13	0-5 mo.	.095090	.230700	.103580
			6-11 mo.	1.175530	3.099790	1.266850
			12-23 mo.	2.249120	4.864760	2.432540
			2-65+ yr.	3.898690	8.193070	4.138540
Stannous chloride NAS 0213	08 Proced frut (R)	*	0-5 mo.	.039010	.104580	.046060
			6-11 mo.	.429940	1.070700	.507640
			12-23 mo.	.834950	1.657510	.985880
			2-65+ yr.	.981890	2.079980	1.159340

Table 18 (104)

NAS/NRC Survey of GRAS Substances - Stannous Chloride*

No. of reports to NAS 1960/1970	Lbs. reported to NAS 1960	1970	Total 1970 lbs. reported to NAS	No. of reports to FEMA	Lbs. reported to FEMA only	Total 1970 lbs.
9/14	4,000	27,294	40,581	----	----	40,581

*Figures represent 60-70% of actual poundage added to nations' food supply.

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The Uptake of Lead and Tin by Fat and Fatty Foods in Tin Plated Cans.*

by

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* Dedicated to Prof. Leopold Schmid, Vienna, on his 70th birthday

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Introduction

The tin plate currently used is for the most part electroplating. The tin applied in this process and the grain tin used for hot tin plating have a lead content of less than 0.5%, indeed as a rule less than 0.1%, and thus meets the specifications of the lead-zinc law (1,2,3). For the soldering of grooved or overlapping can bodies and for closure of the filling hole of oil and milk cans outside solder is used with a lead content which in some cases is more than 50%. In the soldering process technologically unavoidable amounts of this

outside solder may find their way inside the can (1). It is important for evaluation of health aspects to determine how much lead and tin in taken up by the contents of the cans from the solder and the tin plating. The reciprocal effect between tin plated cans and an aqueous content has been investigated in various earlier works (2, 4-8). Quantitative studies on the uptake of heavy metals by the aqueous contents of tin plated cans have been described by Jakobsen and Mathiessen (7), Kogan and Rochlina (9), Bergner and Miethke (1). Engst and Woggon (10) found no significant increase of lead in condensed milk, even when the solder for the filling hole contained 50% lead, but they found a slightly increased lead content when larger quantities of an outside solder with 60% lead found their way inside the cans.

There are few data on fatty foodstuffs in tin plated cans; Szarski (11) among others has studied their lead content and regards solders with 60-70% lead as suitable for meat cans, since the content of the canned material was less than 2 mg/kg. Lampitt and Rooke (12) have reported on sardines in oil. They found up to 90 mg/kg lead, because the fish, befor canning, had been treated on tin plated screens with a high lead content. Table 1 shows the lead content of some oils and fat-containing foods based on the literature.

Even less attention has been devoted to the tin content of canned foods rich in fats. Owe (17) has found tin contents

of up to 1.4 g/kg in poor quality canned meat and fish. Bagnal (18) has reported two anchovy samples with 760 and 785 mg/kg tin, Williams has reported anchovy pastes with 570 to 960 mg/kg tin in 5 out of 70 samples examined. According to Bolko (20) in the course of 5 years an increase in the tin content of canned beef from 16 to 93 mg/kg is to be expected.

The present work deals with the lead and tin uptake of cooking oil and canned foods rich in fat, such as lard, liver wurst, pork and beef in commercial tin plate cans during a storage period of 1 year.

Findings in Commercial Samples.

The study of a number of commercial samples of cooking oil (Table 2), lard (Table 3) and liver wurst (Table 4), according to methods described below, showed, as anticipated, that the foodstuffs, which varied in their fat and water content, revealed different metal contents. However, no rules as to the uptake of metals could be deduced from the values found, since the initial values as well as the conditions of manufacture and storage of the samples were not known. The values also varied from one can to another. Nevertheless, the heavy metal content appeared to be higher in the canned material when outside solder penetrated inside the can and when the inside of the cans was not lacquered.

We were struck by the fact that the lead and tin contents of liver wurst and liver wurst-like pastes in small, oval,

flat cans with roll-up tops (Table 4) were relatively high, apparently because solder was 1 to 1.5 mm wide at the folded seam and always distinctly penetrated inside the cans from the roll-up covers causing an unfavorable content:surface ratio. Thus this type of can appears to be unsuitable for the packaging of fatty foodstuffs.

However, the lead content of the foodstuffs studied was invariably below the values cited in the literature and did not exceed the permissible maximum values given by Truffart (21) for fatty oils (2.0 mg/kg), meat products and canned meats (2.5 mg/kg). Nor was the maximum quantity of 250 mg/kg tin, generally regarded as permissible from the health standpoint, exceeded (Massy, 14, Dixon, 22).

Increase of Lead and Tin during Storage

In order to follow the increase of lead and tin under known conditions, refined soybean oil (Table 5), lard (Table 6) and liver wurst (7) were studied in an experiment lasting one year.

Refined soybean oil scarcely takes up lead or tin during one year of storage from polished grooved cans with filling holes.

However, a greater uptake of lead from the solder must always be anticipated if the cans are damaged during transport or storage, when the solder originally joining the body and inverted lid is exposed over a large surface, so that the

contents of the can come into greater contact with it. Fig. 3 shows a cross section of the severely dented can no. 2 from Table 6. Added to this there is the fact that at these damaged points the surface of the solder was full of fissures and much enlarged in contrast to the smooth surface of melted solder.

This can also be demonstrated experimentally: parts of can 2 (Table 6) were placed in 10 ml cooking oil in the presence of air oxygen. After 4 weeks of storage at 37° the following lead contents were found:

Can portion from Fig. 1: 6.2 µg lead/10 ml oil

Can portion from Fig. 2: 45 µg lead/10 ml oil

Can portion from Fig. 3: 140 µg lead/10 ml oil

Lard and liver wurst in lacquered, overlappingly soldered cans showed a somewhat higher lead and tin uptake than soybean oil. This can be attributed on the one hand to the distinct entry of solder at the seam of these cans and on the other to the greater aggressiveness of these materials.

From findings obtained so far on commercial samples of lard and liver wurst and from the storage experiments with cans with soldered overlapping seams filled with the same foods, it may be concluded that the essentially slight increase in lead and tin content could be attributed to the penetration of outside solder, since these cans were almost all lacquered. It was therefore of interest to study the behavior of lacquered grooved cans, in which there was only a very slight possibility of contact between the contents and the outside solder, notably

at points of fissure and those points at which the solder penetrated through the groove by capillary action.

It was found that after one year of storage pork and beef in grooved cans lacqured on the inside (Table 8) had taken up virtually no lead or tin; the heavy metal content was roughly that of the initial values for lard and liver wurst (see tables 6 and 7).

Further experiments were made to determine some causes of lead and tin uptake.

Influence of Free Fatty Acids

Pieces of test sheet metal (c. 2 x 6 cm) of tin, lead and two lead solders (lead content 65% and 98%) were stored for 1 month at room temperature and exposed to air in lard, liver wurst, soybean oil, refined soybean oil with an added 0.1 % oleic acid and in pure oleic acid. About 1/5 of the surface of the sheet metal protruded into the air. Next the loss of weight of the pieces of test sheet metal was determined according to DIN 50905 (Table 9).

The results led to the following conclusions:

1. Tin is scarcely affected by these storage media. Only lard gave rise to a slight and liver wurst to a somewhat greater loss of weight.

2. Lead and lead-containing solders were distinctly affected in all cases. The aggressiveness of liver wurst

here again was more pronounced than that of lard.

The higher the free fatty acid content of the storage medium was, the greater the weight loss.

3. Of the two lead solders, the solder L-PbSn 35 with the higher tin content was less affected.

Figs. 4 and 5 give an idea of the corrosion observed in the test sheet metals.

A large portion of the lead dissolved from the lead-containing test sheetmetal was found again in the solid products of corrosion, the rest went into solution.

The characteristic bands of lead linoleate and lead stearate appeared in the IR spectrum of the solid products of corrosion recrystallized from alcohol. Similarly the diffraction lines or lattice constants of lead stearate and lead linoleate were found in the Debye-Scherrer diagram.

Stearic acid, linolic acid, linoleic acid, lauric acid, myristic acid, palmitic acid and oleic acid could be detected by gas chromatography in the corrosion products.

Influence of Oxygen Peroxide

Peroxide was produced in 100 ml soybean oil by introducing oxygen at elevated temperatures. After achieving peroxide values of c. 50 to 100 (according to Wheeler), pieces of lead sheet metal (2 x 6) were suspended in the oil and further oxidation was blocked by introducing nitrogen.

Table 10 clearly shows the dependence of the increase of lead in an oil upon its peroxide content.

The production of peroxide could be prevented by filling under vacuum or protective gas, which not only reduces the uptake of heavy metals, but also increases preservability. However, this probably would only be appropriate in special cases.

Influence of Tin Plate Surface

The possibility should also be pointed out that in the manufacture of cans, soluble compounds of soldering metals are produced, for instance oxides or conversion products with the agents used for surface treatment of tin plate or with soldering agents.

However, on the surface of exposed solder in 375 ml cans with filling holes and 400 g meat cans, only 6 µg lead could be detected on the average, in a form easily rubbed off (rubbed off with cotton saturated with alcohol).

Process of Diffusion

It could be assumed that the metal soaps formed upon attack of the can after sterilization would remain localized at the site of attack - in other words at the sites of soldering and tin plating. The findings, however, are contrary to this assumption, since even in solid fats (lard) lead can be found at points further removed from the site of attack. It may be concluded from this that metal soaps diffuse in fatty materials despite their poor solubility.

This was confirmed by a model experiment with pork lard.

The body of tin plated cans was coated with L-PbSn2 solder. The cans were filled with pork lard. After storage for one and two months at 37°C the lead content was determined in concentric layers with a thickness of 1-2 cm.

The diffusion coefficient D was uniformly 1 to 4.10^{-3}, the diffusion of the lead soaps thus followed Fick's law of diffusion (23).

Lead, Tin and Lead Solder Potentials

The following can be seen from the curves of the potentials of lead, tin and two lead solders (L-PbSb35 and L-PbSn2) in lard and liver wurst measured against a saturated calomel electrode (Fig. 6 and 7):

1. After a few days tin reveals the lowest potential and has become base. Thus compared to lead and lead solders, tin rapidly becomes the anode in lard and liver wurst, as well as in most foodstuffs with a high water content.

2. Initially the lead potential is lower than that of tin and thereafter remains roughly at the value reached after a few days. Thus within a short time lead becomes nobler than tin.

3. The potentials of the L-PbSn35 and L-PbSn2 solders are practically the same and always between those of pure lead and pure tin.

It can be concluded from the course of the potentials

of the test electrodes that tin, lead and lead solder behave the same in fatty materials such as lard and liver wurst as in an aqueous medium (Bergner and Miethke, 1). This means that these materials initially attack lead and tin to the same degree, but later essentially only tin, provided there are roughly the same lead and tin surfaces present. In practice lead solder in lacquered cans will confront a large tin anode as cathode over a small surface. In lacquered cans conditions are different, since only the more or less small surface of inwardly penetrating lead solder comes into contact with the can contents, yet in practice the quantities of lead and tin taken up always remain low, even in unfavorable cases.

Determination of microgram quantities of lead and tin in fats and fatty materials

I. Preparation of sample materials.

Wet and dry ashing are used for the preparation of fat and fatty materials for determination of traces of heavy metals.

Wet ashing with sulfuric acid, nitric acid or perchloric acid is difficult and cumbersome, however, in case of large quantities of fat. Furthermore, disturbing quantities of metals can be introduced with the chemical substances used.

Dry ashing is the method most frequently described and studied. Therefore attention was directed principally toward this method or preparation.

According to Absom and Lipscomb (24) one must anticipate a loss of 20% without appropriate precautionary measures in lead determination of material poor in ash at ashing temperatures of 500°C, while in the presence of sulfuric acid at this ashing temperature a good lead yield is found in the "sulfate ash". Ashing can be accelerated by addition of magnesium nitrate solution or magnesium carbonate solution.

Tin compounds are scarcely volatile at temperatures around 500°C. After modification of a method described by Horeczy et al. (25) our procedure was as follows.

Reagents:

(1) Concentrated sulfuric acid, pro anal.

(2) Ultrapure sulfuric acid

(3) Magnesium nitrate, 1% solution in 96% ethanol.

Procedure

100 g sulfuric acid (1) was added to 100 g samples (oil, lard, etc.) in quartz dishes. This was heated on the sand bath for 8 hours at c. 300°C.

Next the quartz cup was heated further on an asbestos wire net with a clay triangle beneath it until the sample was completely charred. After adding 1 ml magnesium nitrate solution (3) calcining at white heat continued for 5 hours at 460°C in the muffle furnace.

If lead traces are to be determined from the ash, ultrapure (2) sulfuric acid must be used.

Extraction of Lead from Cooking Oil

Various authors have referred to the possibility of extracting traces of heavy metals from fatty oil.

Vizern and Guillot (26) heated the fat dissolved in petrol-ether with acetic acid and then precipitated the lead present in the form of acetate with alcohol.

Barney (27) extracted with sulfuric acid and a mixture of hydrochloric acid, acetone and water.

Täufer and Barthel (28) use trichloracetic acid for extraction. Extraction was also successfully achieved with nitric acid, particularly since nitric acid is commercially available "dithizone-pure".

Reagents:

(1) Nitric acid, tested with dithizone

(1a) Nitric acid 1 + 3 (c. 10%)

(3) Sulfuric acid, ultra pure.

Procedure:

100 to 500 ml oil is mixed in a polyethylene flask purified with dithizone with 100 ml nitric acid (1a) and vigorously shaken overnight on a shaker.

After sedimentation and separation of the nitric acid in the separating funnel, the substance is twice shaken by hand with 50 ml double distilled water.

The nitric acid extracts are collected in a 500 ml Kjeldahl flask, mixed with 2 ml ultrapure sulfuric acid (3)

and heated until the nitric acid is distilled off and the sulfuric acid becomes colorless. After cooling 10 ml double distilled water is added, next evaporation takes place, and the process is repeated once more so as to remove the nitric acid without residue. The cooled sulfuric acid solution is mixed with c. 100 ml double distilled water, the pH is adjusted to 9.5 with 14-n ammonia and the lead is extracted as described below with dithizone solution.

II. Micro Lead Determination

Of the methods of lead determination described by Iwantscheff (30) we used spectral photometry of the mixed color from lead dithizonate and free excess dithizone.

The reagents, purification of reagents, and the determination were essentially the same as those described by Bergner and Kiethke (1). In addition, the following is required: 10% diammonium hydrogen citrate solution and 5% sodium carbonate solution.

The ash is first evaporated once by fuming with 5 ml bromine-hydrobromic acid mixture. The residue, covered with a cover glass, is boiled for 1 hour on the sand bath with 25 ml sodium carbonate solution, so as to convert calcium sulfate into calcium carbonate. After cooling, 6-n hydrochloric acid is carefully added until the reaction is acid, after which 10 ml diammonium hydrogen citrate solution is added to avoid precipitation of magnesium- or alkaline earth phosphates. After

neutralization with 14-n ammonia the procedure follows the data given in the literature.

The mean blank value of all purified reagents used in the preparation was 1.5 µg lead. The standard deviation s was 0.3162 with a variance of 9.5%. With a statistical certainty of 99.7% the limit of detection was 2.2 µg lead/25 ml*.

In a range of concentration of 2 to 8 µg lead/25 ml the mean error was 30%.

If lead was extracted from oil with nitric acid and next determined by the method described, the mean blank value of the reagents was 0.5 µg lead. With a standard deviation of the blank values of s = 0.076, the variance of the values was 15.2%. With a statistical certainty of 99.7%, the limit of detection was 1.5 µg lead/25 ml*.

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* Measurement in a 1 cc cuvette.

III. Micro Tin Determination

To determine microgram quantities of tin, according to Koch and Koch-Dedic (29) it is possible to use phenylfluorone rather than dithiol, the former being more sensitive and less subject to disturbance and having a limit of detection of c. 0.2 µg tin/ml, although in this case the limit of detection is strongly dependent on the pH value of the solution used in photometry. Ölschläger (31) and Luke (32) in particular have worked on the determination of microgram quantities of tin.

Taking these works into account, the tin determinations were carried out according to the following procedure:

Reagents:

(1) Phenylfluorone solution: 30 mg phenylfluorone is dissolved in a small amount of methanol, poured over into a 250 ml measuring flask, mixed with 1 ml HCl (2) and filled with methanol up to the mark.

(2) 36% Hydrochloric acid

(3) 10% hydroxylaminesulfate solution in double distilled water

(4) Methanol pro anal.

(5) Double distilled water

(6) Sulfuric acid, concentrated, at least 96%, pro anal.

(7) Hydrochloric acid (6-n) distilled in a pyrex distillation apparatus.

(8) Acetic acid ethylester, pro anal.

(9) L-ascorbic acid, pro anal.

(10) Potassium rhodanide solution, 10% in double distilled water

(11) Hydrogen peroxide, 30%

(12) Sodium carbonate solution, 10%, aqueous.

For the tin determination the material is ashed with sulfuric acid, pro anal.,as described in section I.1.

For conversion of the calcium sulfate produced upon ashing of material rich in calcium, the ash calcined at white heat is boiled with 25 ml sodium carbonate solution (12) on the sand bath for 1 hour. The residue is acidified with hydrochloric acid (2)

and poured over with 20 ml double distilled water into a 250 ml separating funnel. Next 5 ml potassium rhodamide solution (10) and a spatula-tip of ascorbic acid (9) are added, as a result of which the lightly red colored solution assumes a weak rose tone in 1 or 2 min, while occasionally changing back. After the addition of 20 ml acetic acid ethylester (8) the substance is vigorously shaken for 3 min, the aqueous phase is decanted into a 100 ml Erlenmeyer flask and the light red colored ester into a 100 ml glass evaporation vessel.

Shaking out with 20 ml ester is repeated twice more. Next the combined extracts are evaporated until dry over a lightly boiling water btah. The residue is taken up in 1 ml concentrated sulfuric acid (6) and after addition of 20 drops hydrogen peroxide placed in the drying chamber at a temperature of 120°C for 30 min covered with a cover glass. Next 1 ml hydroxylamine sulfate solution (3) is added and the vessel is left in the drying chamber for a further 30 min.

After rinsing the cover glass and pouring on methanol in a 25 ml measuring flask, 5 ml phenylfluorone solution (1) is added and the flask is filled with methanol up to the mark.

After standing for 2 hours in the dark, measurement takes place on the Elko in the 1 cc cuvette, filter S 51 against double distilled water.

The phenylfluorone solution used for coloring keeps for days, but it is nevertheless recommended to determine the blank value for each measurement.

Range of Concentrations

Up to a concentration of 11 µg/25 ml the calibration points are on a straight line. Higher values diverge somewhat. Measurement in the concentration range from 2 to 11 µg/25 ml is recommended.

Evaluation of the Process

The mean blank value of the reagents used for the preparation was 0.56 µg tin. The standard deviation s was 0.0319 with a variance of 14.5%. The limit of detection was 1 µg tin/25 ml (1 cc cuvette).

In a range of concentration of 2 to 11 µg/25 ml the mean error was 10%.

Summary

The uptake of lead and tin from the oils and fatty foods studied in commercial tin plated cans was less than 1 mg/kg, with occasionally considerable variations between individual cans, under storage conditions described below. Thus the maximum quantities of lead and tin permissible from the health standpoint were not exceeded.

Refined soybean oil in polished, grooved cans with filling holes took up an average of 20 µg Pb/kg in 12 months. The uptake of tin during the same period was c. 10 µg/kg. A higher lead content was observed in oil, in some cases more than 100 µg/kg, in dented cans, in which the soldered joint between the body and inverted lid had been torn.

Only 10 to 20 µg/kg Pb/kg and 25 to 35 µg/kg Sn were found in pork and beef from lacquered grooved cans.

Lard and liver wurst in lacquered overlappingly soldered cans showed a somewhat higher uptake of lead and tin (60 to 70 µg Pb/kg, 40 to 50 µg Sn/kg.

In oval flat cans with a roll-up lid the contact with the solder is apparently particularly pronounced and for this reason the lead and tin content of the liver wurst-like pastes packaged in these is the highest (200 to 800 µg Pb/kg, 20 to 60 µg Sn/kg).

As was shown by model experiments, predominandy fatty acid salts of lead are formed upon uptake of lead from fat and fatty foods. The intensity of the attack increases with rising acid and peroxide content. Tin and solder L-PbSn35 were less strongly attacked than lead and solder L-PbSn2. The metal soaps do not remain at the site of their production, but diffuse into the can content. It was shown that this diffusion follows Fick's law of diffusion.

Specifications for the preparation of the sample material (sulfate ash or nitric acid extraction) and for the determination of lead (dithizone method) and of tin (phenylfluorone method) are given. The limit of detection for Pb, according to the method of preparation, is 1.5 to 2.2 µg/25 ml measured in a 1 cc cuvette, with a range of variation of 30%. The limit of detection for Sn^{2+} is 1 µg/25 ml measured in a 1 cc cuvette, with a range of variation of 5%.

Figures

1. Intact joint of floor and body of a can with a filling hole.

2. Incipient detachment of soldering at the floor and body due to slight dent.

3. Pronounced detachment of soldering at the floor and body due to severe dent.

4. Test piece of lead sheet metal in soybean oil. Air/oil border line. 40-fold magnification. Intercrystalline attack.

5. Piece of test sheet metal of L-PbSn35 solder in oleic acid. Localized corrosion at point of immersion. 40-fold magnification.

6. Course of potentials in lard. 1. Lead. 2. Tin. 3. Solder of 65% Pb/35% Sn. 4. Solder of 98% Pb/2% Sn.

7. Course of potentials in liver wurst.

Tables.

1. Lead content of foodstuffs. 1. Author. 2. Type of food. 3. Lead content in mg/kg. 4. Peanut oil. 5. Olive oil. 6. Cooking oil. 7. Oils, fats. 8. Sausage for cooking. 9. Beef. 10. Pork. 11. Cooked meat. 12. Sausage. 13. Meat products.

2. Commercial cooking oils (Organolept. findings; normal in all cases). Abbreviations: POZ: Peroxide value; Ald: aldehyde according to Schiff. 1. Commercial name. 2. Lead. 3. Tin. 3a. Acid value. 4. Peroxide value. 5. Aldehyde. 6. Age. 7. Type of can. 8. Olive oil. 9. Pure plant oil. 10. Reconstituted sunflower oil. 11. Delicatessen peanut oil. 12. Sunflower oil pressed cold. 13. unknown. 14. less than 6 months. 15. 5 months.

16. weakly positive. 17. less than 6 months. 18. distinctly positive. 10. 10 weeks. 20 less than 6 months. 21. unknown. 22. Can with filling hole, overlappingly soldered, no entry of solder. 23. Solder in the filling hole easily entered inside can, overlappingly soldered. 24. Can with filling hole, overlappingly soldered, no entry of solder, slightly dented. 25. Square can, folded seam, no entry of solder. 26. Can with filling hole, folded seam, no entry of solder. 27. Can with filling hole, overlappingly soldered, slight entry of solder. 28. Square can, folded seam, slight entry of solder.

3. Commercial lard. 1. Commercial name. 2. Lead. 3. Tin. 4. Fat. 5. Water. 6. Acid value. 7. Peroxide value. 8. Aldehyde. 9. Approximate age. 10. Type of can. 11. Organoleptic finding. 12. Lard. 13. weakly positive. 14. unknown. 15. 4 months. 16. 2 years. 17. Overlappingly soldered, not lacquered, no entry of solder. 18. Overlappingly soldered, not lacquered, no entry of solder. 10. Overlappingly soldered, no entry of solder, pronounced marbleization. 20. Overlappingly soldered, not lacquered, slight entry of solder. 21. Overlappingly soldered, lacquer detached in places, pronounced marbleization. 22. Overlappingly soldered, distinct entry of solder. 23. Overlappingly soldered, not lacquered, distinct entry of solder, pronounced marbleization. 24. Originally lacquered, lacquer corroded, pronounced rusting, considerable entry of solder. 25. Satisfactory. 26. Bacterial spoilage.

4. Liver wurst and liver paste in oval flat cans, commercial. 1. Name of product. 2. Lead. 3. Tin, 4. Type of can. 5. Organoleptic findings. 6. French Pork liver paste. 7. Burgundy Country liver wurst. 8. Burgundy liver wurst. 9. Liver paste, Strassburg type. 10. Entry of solder at seam and lid. 11. Entry of solder at folded seam and lid, distinct. 12. Distinct entry of solder. 13. Distinct entry of solder. 14. Satisfactory. 15. Slightly metallic, but not spoiled.

5. Influence of time of storage on the Pb and Sn contents and on some characteristic values of cooking oil (A: initial value in polyethylene bottle). 1. Duration of storage. 2. Can number. 3. Lead. 4. Mean value. 5. Tin. 6. Acid value. 7. Peroxide value. 8. Aldehyde. 9. Type of can. 10. Organoleptic finding. 11. Light yellow, clear, satisfactory. 12. 1 week. 13. Undamaged. 14. Strongly dented. 15. Satisfactory. 16. 3 months. 17. Almost undamaged. 18. Undamaged. 19. Dented. 20. Damaged at inverted floor portion. 21. Severe damage, running out in part. 22. Undamaged. 23. Slight rancid after-taste. 24. weakly positive. 25. Slight rancid odor and taste. 26. Slightly altered, but not spoiled.

6. Influence of time of storage on the Pb and Sn content and on some characteristic values of lard (A: initial value in preserving glass). 1. Duration of storage. 2. Can number. 3. Lead. 4. Mean value. 5. Tin. 6. Acid value. 7. Peroxide value. 8. Aldehyde. 9. Properties of cans. 10. Organoleptic findings. 11. Weakly positive. 12. Upon opening of glass, slight odor of hydrogen sulfide. 13. Satisfactory. 14. strongly positive.

7. Influence of time of storage on the Pb and Sn content and some characteristic values of liver wurst (A = initial value in preserving glass). Headings as in Table 6.
1. Distinct odor and taste of liver, slightly bitter aftertaste (Frozen liver), distinct taste of boiling.

8. Pb and Sn content of pork and beef after one year of storage in lacquered grooved cans. 1. Type of meat. 2. Lead. 3. Mean value. 4. Tin. 5. Fat. 6. Water. 7. Qualities of cans and findings. 8. Pork. 9. can 1. 10. can 2. 11. Beef. 12. can 1. 13. can 2. 14. satisfactory.

9. Corrosion phenomena in tin, lead and lead solders. 1. Storage medium. 2. Type of test sheet metal. 3. Loss of weight per 100 cc. 4. Visible changes in storage medium. 5. Changes in test sheet metal, macroscopic and microscopic. 6. Soybean oil. 7. Soybean oil + 0.1% oleic acid. 8. Oleic acid, pure. 9. Lard. 10. Liver wurst. 11. Tin. 12. Lead. 13. None. 14. Strong sediment. 15. Slight sediment. 16. Distinct intercrystalline attack at air/oil line of contact. 17. Slight localized corrosion. 18. Black coating at air/oil line of contact. 19. Strong corrosion, voluminous precipitate, distinct localized corrosion at air/oil line of contact. 20. Slight deposit. 21. White coating at air/oleic acid line of contact, strong adhesion of corrosion products, intercrystalline attack. 22. Voluminous precipitate, depositedat air/oleic acid line of contact, distinct localized corrosion. 23. Pronounced deposition of corrosion products, distinct localized corrosion at air/oleic acid line of contact. 24. Barely visible corrosion, very slight localized

corrosion. 25. Distinct localized corrosion at air/medium line of contact. 26. Distinct localized corrosion at air/medium line of contact. 27. Localized corrosion at air/medium line of contact. 28. Slight localized corrosion and intercrystalline attack at air/medium line of contact. 29. Distinct localized corrosion at air/medium line of contact and immersed portion. 30. Distinct localized corrosion at air/medium line of contact. 31. Distinct localized corrosion at air/medium line of contact and immersed portion.

10. Influence of the peroxide content of an oil and the Pb content upon storage. 1. Peroxide value according to Wheeler, at the beginning. 2. After 1 month. 3. Acid value. 4. At the beginning. 5. after 1 month. 6. mg Lead in 100 ml after 1 month.

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Zur Blei- und Zinnaufnahme von Fetten und fettreichen Lebensmitteln in Weißblechdosen*

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Einführung

Die heute gebräuchlichen Weißbleche sind überwiegend elektrolytisch verzinnt. Das hierbei abgeschiedene Zinn und das zum Feuerverzinnen verwendete Feinzinn weist einen Bleigehalt von unter 0,05% meist sogar unter 0,01% auf und entspricht damit den Anforderungen der Bleifolienlieferanten (1, 2, 3). Zur Verlötung der gefalzten oder überlappten Dosennähte sowie zum Verschließen der Fülloch von Öl- und Milchdosen werden Außenlote mit einem Gehalt von teilweise 50% Blei verwendet. Beim Lötvorgang können technisch nicht vermeidbare Anteile vom Außenlote in das Innere der Dosen gelangen (4). Für die gesundheitliche Beurteilung ist maßgebend, wieviel Blei und Zinn vom Inhalt der Konservendosen aus dem Lot und der Verzinnung aufgenommen wird. Die Wechselwirkung zwischen Weißblechdosen und wäßrigen Füllgut ist in verschiedenen älteren Arbeiten untersucht (2, 4—8). Quantitative Untersuchungen über die Schwermetallaufnahme in fettigen Füllgut in Weißblechdosen beschreiben u. a. JAKOBSEN und BERNASEK (7), KOCAN u. ROSMUSSEN (9), BERNASEK u. MERRIGS (1), ESSER u. WOLFF (10) sehr ausführlich. Sie fanden keine signifikante Bleiaufnahme, auch wenn das Fülloch 50% Blei enthielt, jedoch fanden sie einen leicht erhöhten Bleigehalt, wenn größere Mengen eines Außenlotes mit 50% Blei in das Dosenintere gelangen.

Die fettfreien *Lebensmittel* aus Weißblechdosen bieten wenig Angaben vor; u. a. untersuchte FRANZ (11) deren Bleigehalt; er fand Werte mit 60 bis 70% Blei für Obstdosen für geeignet, da der Bleigehalt des Füllgutes unter 2 mg/kg lag. Über Sardinen in Öl berichteten JAMBOTT u. KOSSE (12). Sie fanden bis zu 90 mg/kg Blei, wenn die Fische vor dem Einlegen auf Rosten behandelt worden waren, die mit einer stark bleihaltigen Verzinnung überzogen waren. Bei Ersatz der Rostgarnitur enthielten einige Öle und fetthaltiger Lebensmittel nach Literaturangaben.

Sehr wenig Beachtung ist dem Zinngehalt eingedoster fettreicher Lebensmittel geschenkt worden. So findet DEWE (17) in Fleisch- und Fischkonserven schlechter Qualität Zinngehalte bis 14 mg/kg. BANSAL (18) berichtet über zwei Ananasproben mit 700 und 785 mg/kg Zinn. BRIANI (19) über Anchovispasten mit 570 bis 600 mg/kg Zinn in 3 von 30 untersuchten Proben. VOLBORTH (20) ist in Rindfleischkonserven im Laufe von 5 Jahren mit einem Anstieg des Zinngehaltes von 16 auf 81 mg/kg zu rechnen.

Die vorliegende Arbeit beschäftigt sich mit der Blei- und Zinnaufnahme von Speiseöl und von fettreichen Füllgütern, wie Schinkenfleisch, Leberwurst, Schweine- und Rindfleisch in handelsüblichen Weißblechkonservendosen während einjähriger Lagerung.

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*Herrn Prof. Dr. LEUCHS zum 70. Geburtstag gewidmet.

**Auszug aus U. HÜTT: Zur Blei- und Zinnaufnahme von Fetten und fetthaltigen Lebensmitteln in Weißblechkonservendosen. Dissertation TH Stuttgart 1957.

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Tabelle 1. *Bleigehalte einiger Lebensmittel*

Autor	Art des Lebensmittels	Bleigehalt in mg/kg
Sheffer, H. (12)	Erdnußöl	0,90
Sheffer, H. (13)	Olivenöl	0,71
Sheffer, H. (13)	Speiseöl	1,25
Mazur, R. (14)	Öle, Fette	0,20
Kirch u. Mitarb. (15)	Kochwurst	0,10—1,00
Breyer-Heusser (16)	Rindfleisch	0,16
Breyer-Heusser (16)	Schweinefleisch	0,07
Breyer-Heusser (16)	Fisch, geräuchert	Spuren bis 0,03
Mazur, R. (14)	Wurst	0,27
Mazur, R. (14)	Kleinkinderwaren	2,5

Tabelle 2. *Speiseöle aus dem Handel* (ungemischt). Befund: stets eisenanlöst. Abkürzungen: PZ, Peroxidzahl; Ald., Aldehyde nach Scherr)

Handelsbezeichnung	Blei µg/kg	Zinn µg/kg	n_D^{20}	SZ	PZ	Ald.	Alter	Dosenbeschaffenheit
Rohöl	7,5	0	1,475	0,20	2,8	0	unbek.	Fülleichlöcher, überlappt und verlötet, kein Lotübertritt
Reines Pflanzenöl	0	0	1,475	0,12	1,6	0	unter 6 Mon.	Fülloeichlöcher, überlappt verlötet, kein Lotübertritt
Reines Sonnenblumenöl	0	0	1,475	0,10	3,2	0	3 Monate	Lot im Falz leicht nach innen eingetreten, überlappt gelötet
Erdnußöl	17,5	0	1,475	0,24	2,4	schwach positiv	unter 6 Mon.	Fülloeichlöcher, überlappt verlötet, kein Lotübertritt, keine Verbeulung
Pflanzenöl	0	0	1,478	0,14	3,1	0	unter 6 Mon.	Fülloeichlöcher, überlappt verlötet, kein Lotübertritt
Hoffmann-La Roche Erdnußöl	0	10,0	1,472	0	3,6	deutl. positiv	10 Wochen	viereckige Löcher, Falzmaß, kein Lotübertritt
Reines Pflanzenöl	87,5	14,0	1,475	0,20	4,4	0	4 Wochen	Fülloeichlöcher, Falzmaß, kein Lotübertritt
Reines Pflanzenöl	25,0	0	1,475	0,27	0,8	0	unter 6 Mon.	Fülloeichlöcher, überlappt verlötet, Lotübertritt gering
Hydrogeniertes Sonnenblumenöl	17,0	0	1,475	0,10	5,1	0	unbek.	viereckige Löcher, Falzmaß, geringer Lotübertritt

Befunde an Proben aus dem Handel

Die Untersuchung einer Anzahl aus dem Handel entnommener Proben Speiseöl (Tab. 2), Schmalzfleisch (Tab. 3) und Leberwurst (Tab. 4) nach den später angegebenen

Tabelle 3. *Schmalzfleisch aus dem Handel*

Schmalzfleisch	Bezeichnung 1	Blei µg/kg 2	Zinn µg/kg 3	Fett % 4	Wasser % 5	N-haltige Stoffe %	SZ 6	POZ 7	Jod 8	Alter 9	Dosenbeschaffenheit 10	Organoleptischer Befund 11
Schmalzfleisch (300.6g)		30,0	100	18,2	40,9	10,0	0,3	0	0	10 Monat.	überlappt gefalzt, nicht lackiert, kein Lackübertritt	25 einwandfrei
(300.0g)		0	40,0	40,1	39,0	10,0	0,3	0	0	15 4 Mon.	überlappt gefalzt, lackiert, kein Lackübertritt	einwandfrei
Schmalzfleisch (290.4g)		0	30,0	38,5	34,1	9,8	0,3	0,2	0	16 2 Jahre	überlappt gefalzt, nicht lackiert, kein Lackübertritt	einwandfrei
(340g)		0	45,0	36,1	32,0	10,5	0,3	0	0	1 Jahr 17	überlappt gefalzt, kein Lackübertritt, stark marmoriert	einwandfrei
(289g)		30,0	14,0	36,3	40,5	9,8	0,3	0,2	13 schwach positiv	4 Mon. 20	überlappt gefalzt, nicht lackiert, schwacher Lackübertritt	einwandfrei
(325g)		28,0	97,0	31,3	39,0	9,4	1,2	0	0	3 Mon. 21	lackiert, Lack stellenweise abgelöst, stark marmoriert	einwandfrei
(331.0g)		30,0	20,0	63,3	31,0	7,0	0,30		0	3 Mon. 22	überlappt gefalzt, nicht lackiert, Lackübertritt deutlich	einwandfrei
(346.0g)		126	82,0	42,5	30,2	8,2	0,7	0,2	schwach positiv	2,5 Mon.	überlappt gefalzt, nicht lackiert, Lackübertritt deutlich	einwandfrei
(246.5g)		84,0	200	38,0	31,2	7,0	1,2	0	0	1,5 Jahre 23	überlappt gefalzt, nicht lackiert, Lackübertritt deutlich, stark marmoriert	einwandfrei
(196.5g)		25,0	100	38,2	31,1	9,0	0,8	0	0	1,5 Jahre	überlappt gefalzt, nicht lackiert, Lackübertritt deutlich	einwandfrei
(292.6g)		320	210	71,7	28,0	0,4	1,3	0	0	2 Jahre 24	unpräparatlich lackiert, Lackierung durchgefressen, stark angerostet, starker Lackübertritt	26 bakteriell verdorben

Tabelle 4. *Leberwurst und Leberpasteten in ovalen Flachdosen aus dem Handel*

Bezeichnung 1	2 Blei µg/kg	3 Zinn µg/kg	4 Dosenbeschaffenheit	5 Organoleptischer Befund
6 Französische Schweineleberpastete (62 g)	203	17,0	10 Lotübertritt an Nahtstelle und Deckel	14 einwandfrei
7 Burgunder Landleberwurst (82,5 g)	225	18,5	11 Lotübertritt an Falznaht und Deckel deutlich	15 leicht metallisch, jedoch noch nicht verdorben
8 Burgunder Leberwurst (84,0 g)	530	43,0	12 Lotübertritt deutlich	einwandfrei
9 Leberpastete Straßburger Art (77,5 g)	820	44,0	13 Lotübertritt deutlich	einwandfrei

Methoden ergab erwartungsgemäß, daß die in ihrem Fett- und Wassergehalt verschiedenen Lebensmittel unterschiedliche Metallgehalte zeigen. Aus den gefundenen Werten können jedoch keine Gesetzmäßigkeiten der Metallaufnahme abgeleitet werden, da die Ausgangswerte sowie die Herstellungs- und Lagerungsbedingungen dieser Proben nicht bekannt sind. Auch schwanken die Werte von Dose zu Dose stark, jedoch scheint der Schwermetallgehalt im Füllgut dann höher zu sein, wenn Antimon bei in das Innere der Dosen eingedrungen ist und wenn die Dosen innen nicht lackiert sind.

Es fällt aber auf, daß die Blei- und Zinngehalte von Leberwurst und leberwurstartigen Pasteten in kleinen ovalen Flachdosen mit Abreißdeckel (Tab. 4) verhältnismäßig hoch sind, offenbar, weil Lot an der Falznaht 1 bis 1,5 mm breit und am Abreißdeckel stets deutlich in das Innere der Dosen eingetreten war und das Verhältnis Inhalt/Oberfläche ungünstig ist. Diese Dosen dürften somit zur Verpackung von fettreichen Lebensmitteln wenig zweckmäßig sein.

Die Bleigehalte der untersuchten Lebensmittel liegen jedoch durchweg unterhalb der in der Literatur angegebenen Werten und überschreiten die von Tarrant (21) angegebenen duldbaren Höchstwerte für fette Öle (2,0 mg/kg), Fleischwaren und Dosenfleisch (2,5 mg/kg) nicht. Ebensowenig wurde die für Zinn allgemein gesundheitlich noch als duldbar angesehene Höchstmenge von 250 mg/kg [Massy (14), Dixon (24)] überschritten.

Blei- und Zinnaufnahme während der Lagerung

Um die Blei- und Zinnaufnahme unter bekannten Bedingungen zu verfolgen, wurden in einem einjährigen Versuch raffiniertes Sojaöl (Tab. 5), Schmalzfleisch (Tab. 6) und Leberwurst (Tab. 7) untersucht.

Raffiniertes Sojaöl nimmt aus blanken, gefalzten Füllochdosen während einjähriger Lagerung Blei und Zinn kaum auf.

Jedoch ist stets dann mit einer größeren Bleiaufnahme aus dem Lot zu rechnen, wenn die Dosen beim Transport oder bei der Aufbewahrung so beschädigt werden, daß das ursprünglich Rippe und Stülpdeckel verbindende Lot in größerer Fläche freiliegt, so daß das Lot gut damit verstärkt in Kontakt kommt. Abb. 1 zeigt das Schnittbild der stark verbeulten Dose Nr. 2 aus Tab. 6. Hinzu kommt noch, daß an diesen Bißstellen die Oberfläche des Lots zerklüftet und somit stark vergrößert war im Gegensatz zur glatten Oberfläche von aufgespachteltem Lot.

Dies läßt sich auch experimentell zeigen: Teile der Dose Nr. 2 (Tab. 6) wurden in Gegenwart von Luftsauerstoff in je 10 ml Speiseöl gelegt; nach 4wöchiger Lagerung bei 37° C wurden folgende Gehalte gefunden:

Dosenteil Abb. 1: 16,2 µg Blei 10 ml Öl,
Dosenteil Abb. 2: 45 µg Blei 10 ml Öl
Dosenteil Abb. 3: 140 µg Blei 10 ml Öl.

Schmalzfleisch und Leberwurst in lackierten, überlappt gelöteten Dosen zeigen eine etwas größere Blei- und Zinnaufnahme als Sojaöl. Dies ist einerseits auf den reichlichen Lösungsmittelzutritt an der Naht in diesen Dosen, andererseits auf die höhere Aggressivität dieser Füllgüter zurückzuführen.

Anschließend bisherigen Befunden an Proben von Schmalzfleisch und Leberwurst aus dem Handel und den für die Lagerversuche mit den gleichen Füllgütern befüllten *verlötet gelöteten* Dosen konnte die an sich geringe Zunahme des Blei- und des Zinngehaltes auf das eingeleitete Außenluft zurückgeführt werden, da diese Dosen fast

Tabelle 5: *Einfluß der Luftzufuhr auf Pb- u. Sn-Gehalte sowie auf einige Kennzahlen von Speiseöl* (A: Ausgangswert in Dihydrolinolsäure)

Lagerung	Dose	Dose Nr.	Blei µg/kg	Mittelwert µg/kg	Zinn µg/kg	Mittelwert µg/kg	SZ	POZ	Ald.	Dosenbeschaffenheit	Organoleptischer Befund
		A	9,0 7,5	8,2	29 31	29,5	1	0	0		beliebig, klar einwandfrei
60d	Woche	1 2	11,2 104		39,0 122		1	0,6	0	unverändert stark verbeult	einwandfrei
6°C											
3	Monate	3 4	11,0 11,5	11,7	22,0 21,0	21,0	0,1	0,2	0	nahezu unverändert	einwandfrei
6	Monate	5 6	10,0 11,7	–	20,0 70,0	–	0,2	7,7 3,1	0	unverändert verbeult	einwandfrei
9	Monate	7 8	36,0 27,5	(32)	33 80	–	0,1	5,0	0	unverändert zum Hohlraum hin beschädigt	einwandfrei
12	Monate	9 10	26,0 65,0	–	47,0 20,0	33,0	0,7	3,2	0	unverändert stark beschädigt, teilweise ausgebeult	einwandfrei
37°C											
3	Monate	11 12	22,5 15,0	18,5	24,0 25,0	24,5	0,2	4,8 3,1	0	unverändert unverändert	leicht ranziger Nachgeschmack
6	Monate	13 14	38,5 38,5	38,5	10,0 15,0	12,5	0,1	3,7	schwach positiv	unverändert unverändert	nicht ausgeprägt ranziger Geruch u. Geschmack
9	Monate	15 16	37,5 75,0	—	17,0 23,0	20,0	0,1	3,5	schwach positiv	unverändert unverändert	leicht verändert, aber noch nicht verdorben
12	Monate	17 18	45,0 10,0	12,5	43,0 47,5	45,2	0,4	5,0	schwach positiv	unverändert unverändert	leicht verändert, aber noch nicht verdorben

Tabelle 6. *Einfluß der Lagerzeit auf Blei-, Zinkgehalte und auf einige Kennzahlen von Schnaps.*
(A: Ausgangswert in Einweckglas)

Lagerdauer	Probe Nr.	Blei	Mittelwert	Zinn	Mittelwert	SZ	DSZ	Abl.	Bernsteinfindlichkeit	Fingerabdruck
		γ/kg		γ/kg						
	A	6,0 6,0	6,0	13,0 13,0	13,0	0,5	0,2	Molybdänblau positiv		
ca. 1 Woche	1 2	6,0 7,0	6,5	13,0 13,0	13,0	0,5	0,2	0	einwandfrei	einwandfrei
37° C										
3 Monate	3 4	10,0 12,0	11,0	20,0 23,0	21,0	0,5	0	0	einwandfrei	einwandfrei
6 Monate	5 6	21,0 28,0	24,5	13,0 13,0	13,5	0,5	0	0	einwandfrei	einwandfrei
9 Monate	7 8	50,0 58,0	57,0	23,0 20,0	22,5	0,5	0	schwach positiv	einwandfrei	einwandfrei
12 Monate	9 10	69,0 33,0	(53)	30,0 29,0	(29)	1,2	0	schwach positiv	einwandfrei	einwandfrei
37° C										
3 Monate	11 12	15,0 18,0	17,5	25,0 25,0	25,0	0,5	0,4	0	einwandfrei	einwandfrei
6 Monate	13 14	34,5 25,0	30,0	25,0 23,0	24,0	0,6	0	0	einwandfrei	einwandfrei
9 Monate	15 16	25,0 10,0	17,5	32,0 20,0	26,0	1,0	0	0	einwandfrei	einwandfrei
12 Monate	17 18	62,0 67,0	64,0	30,0 70,0	(50,0)	1,4	0	stark positiv	einwandfrei	einwandfrei

Abb. 1. Intakte Verbindung von Boden und Rumpf einer Fäulnisbiese

Abb. 2. Beginnende Lösung der Verbindungen Boden und Rumpf infolge leichter Verbeulung

Abb. 3. Starke Ablösung Verbeulung an Boden und Rumpf infolge starker Verbeulung

Tabelle 7. *Einfluß der Lagerzeit auf Pb- u. Sn-Gehalt sowie auf einige Kennzahlen von Leberwurst* (A Ausgangswerte in Klammerkästen)

Sorte	Dose Nr.	Blei µg/kg	Mittelwert µg/kg	Zinn µg/kg	Mittelwert µg/kg	SZ	VZ	Ald.	Dosenbeschaffenheit	Organoleptischer Befund
	A	7,5 13,0	10,0	40,0 44,0	42,0	3,5	1,0	0	-	1 Geruch und Geschmack deutlich n. Leber, leicht bitterer Nachgeschmack (Gefrierleber), deutl. Kochgeschmack
0	Sorte	1 2	12,5 20,0	16,5	30,0 30,0	–	2,7	1,8	2 schwach positiv	einwandfrei einwandfrei
3	Sorte	3 4	10,0 15,0	12,5	40,0 45,0	42,5	3,0	0	0	einwandfrei einwandfrei
6	Sorte	5 6	41,0 40,0	40,5	45,0 45,0	45,0	2,5	0	0	einwandfrei einwandfrei
9	Sorte	7 8	34,0 42,0	38,0	60,0 67,0	72,0	5,2	0	0	einwandfrei einwandfrei
12	Sorte	9 10	45,0 77,0		27,0 81,5		2,7	0	0	einwandfrei einwandfrei
3	Sorte	11 12	13,0 9,0	11,0	35,0 30,0	32,5	3,0	0	0	einwandfrei einwandfrei
6	Sorte	13 14	41,0 23,0	32,0	48,0 48,0	18,0	0,5	0	0	einwandfrei einwandfrei
9	Sorte	15 16	37,5 75,0	–	70,0 85,0	77,0	0,5	0	0	einwandfrei einwandfrei
12	Sorte	17 18	47,5 60,0	–	55,0 45,0	50,0	7,5	0	0	einwandfrei einwandfrei

Tabelle 8. *Pb- und Sn-Gehalt in Schweinefleisch und Hühnerfleisch nach einjähriger Lagerung in kochfertigen Fertigkost*

1 Probeart	2 Blei µg/kg	3 Mittelwert µg/kg	4 Zinn µg/kg	Mittelwert µg/kg	5 Fett %	6 Wasser %	7 Dosenbeschaffenheit u. Sinnenbefund
8 Schweinefleisch 9 Dose 1	20,0 15,0	18	34,0 37,5	36			14 einwandfrei
10 Dose 2	10,0 12,0	11	25,0 27,0	25	18,5	62,0	einwandfrei
11 Hühnerfleisch 12 Dose 1	10,5 22,5	21	25,5 35,5	30			einwandfrei
13 Dose 2	14,0 20,0	17	34,0 38,0	36	42,3	41,0	einwandfrei

Tabelle 9. *Korrosionsuntersuchungen an Zinn, Blei und Bleilot*

Lagermedium	Art des Prüfblechs	Gewichtsverlust je 100 cm²	sichtbare Veränderungen im Lagermedium	Veränderungen am Prüfblech makroskopisch und mikroskopisch
Sojaöl	Zinn	0,0 mg	keine	keine
	Blei	1010,0 mg	keine	an Berührungslinie Luft Öl deutlicher interkristalliner Angriff
	L-PbSn 2	192,0 mg	keine	keine
	L-PbSn35	28,5 mg	keine	geringer Lochfraß
Sojaöl + 0,7% Ölsäure	Zinn	0 mg	keine	keine
	Blei	405,0 mg	starker Bleiseife	schwarzer Belag an Berührungslinie Luft
	L-PbSn 2	260,0 mg	keine	starker Korrosionsangriff, großvolumiger Abschlag, deutlicher Lochfraß an Berührungslinie Luft Öl
	L-PbSn35	38,0 mg	keine	leichte Abtragung
Ölsäure, rein	Zinn	0 g	keine	keine
	Blei	1,3 g	leichter Bleiseife	weißer Belag an Berührungslinie Luft Ölsäure, starke Umbildung des Korrosionsproduktes, interkristalliner Angriff
	L-PbSn2	1,2 g	keine	großvolumiger Abschlag, Abtragung an Berührungslinie Luft Ölsäure deutlicher Lochfraß
	L-PbSn35	1,7 g	leichter Bleiseife	starker Ansatz der Korrosionsprodukte deutlicher Lochfraß an Berührungslinie Luft Ölsäure
Schmalzfleisch	Zinn	3,0 mg	keine	Korrosion kaum sichtbar, geringer leichter Lochfraß
	Blei	30,5 mg	keine	deutlicher Lochfraß an Berührungslinie Luft Medium
	L-PbSn2	28,0 mg	keine	deutlicher Lochfraß an Berührungslinie Luft Medium
	L-PbSn35	35,0 mg	keine	Lochfraß an Berührungslinie Luft Medium
Leberwurst	Zinn	10,7 mg	keine	leichter Lochfraß und interkristalliner Angriff an Berührungslinie Luft Medium
	Blei	108,0 mg	keine	deutlicher Lochfraß an Berührungslinie Luft Medium und eingetauchtem Teil
	L-PbSn2	118,0 mg	keine	deutlicher Lochfraß an der Berührungslinie Luft Medium
	L-PbSn35	50,0 mg	keine	deutlicher Lochfraß an der Berührungslinie Luft Medium und eingetauchtem Teil

lackiert waren. Es war daher interessant, das Verhalten von lackierten *Falz*dosen zu untersuchen, bei denen eine Berührung des Inhaltes mit Außenluft nur sehr beschränkt möglich ist, nämlich an den Auszwickungen und an den Stellen, an denen die Kapillar in den Falz eingedrungen ist.

Es erwies sich, daß Schweinefleisch und Rindfleisch nach einjähriger Lagerung in *lackierten Falzdosen* (Tab. 8) praktisch kein Blei und Zinn aufnahmen; die Lösemetallgehalte liegen irrnähernd bei den Ausgangswerten von Schmalzfleisch und Leberwurst (vgl. Tab. 6 u. 7).

Zur Beurteilung einiger Ursachen der Blei- und Zinnaufnahme wurden weitere Versuche angestellt.

Einfluß von freien Fettsäuren

Prüfbleche (ca. 2 × 6 cm) aus Zinn, Blei und zwei Bleiloten (Bleigehalt: 65% und 98%) wurden 1 Monat lang bei Zimmertemperatur und Luftzutritt in Schmalzfleisch, Leberwurst, Sojaöl, diesem Sojaöl mit 0,1% zugesetzter Ölsäure und in reiner Ölsäure gelagert. Etwa 1/3 der Oberfläche der Bleche ragte in den Luftraum. Anschließend wurde der Gewichtsverlust der Prüfbleche nach DIN 50905 bestimmt (Tab. 9).

Die Ergebnisse lassen folgende Schlüsse zu:

1. Zinn wird von diesen Lagerungsmedien kaum angegriffen. Lediglich Schmalzfleisch wirkt einen geringen, Leberwurst einen etwas größeren Gewichtsverlust.

2. Blei und bleihaltige Lote werden in allen Fällen deutlich angegriffen. Die Aggressivität von Leberwurst ist auch hier ausgeprägter als die von Schmalzfleisch.

Je höher der Gehalt des Lagerungsmediums an freier Fettsäure ist, desto größer ist der Gewichtsverlust.

3. Von den beiden Bleiloten wurde das zinnreichere Lot L-PbSn35 am wenigsten angegriffen.

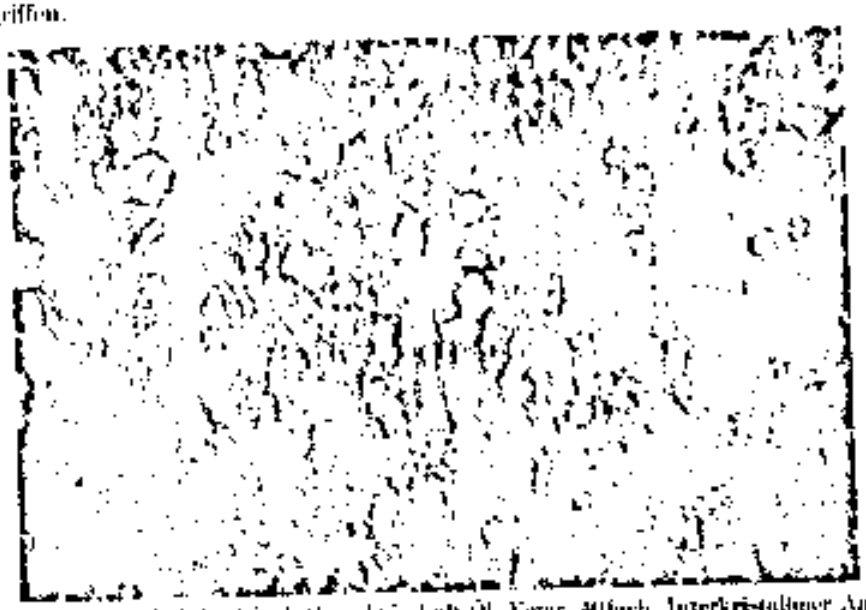

Abb. 4. Bleiprüfblech in Sojaöl, Grenzfläche Luft/Öl. Vergr. 40fach. Interkristalliner Angriff

Einen Eindruck der an den Prüfblechen festgestellten Korrosion geben die Abb. 4 und 5.

Ein großer Teil des aus den bleihaltigen Prüfblechen herausgelösten Bleis wurde in den festen Korrosionsprodukten wiedergefunden; der Rest ist in Lösung gegangen.

Im IR-Spektrum der aus Alkohol umkristallisierten festen Korrosionsprodukte traten die charakteristischen Banden von Bleilinoleat und Bleistearat auf. Es zeigten sich im Debye-Scherrer-Diagramm die Beugungslinien bzw. Gitterkonstanten von Bleistearat und Bleilinoleat.

Gaschromatographisch konnten in den Korrosionsprodukten Stearinsäure, Linolsäure, Linolensäure, Laurinsäure, Myristinsäure, Palmitinsäure und Ölsäure nachgewiesen werden.

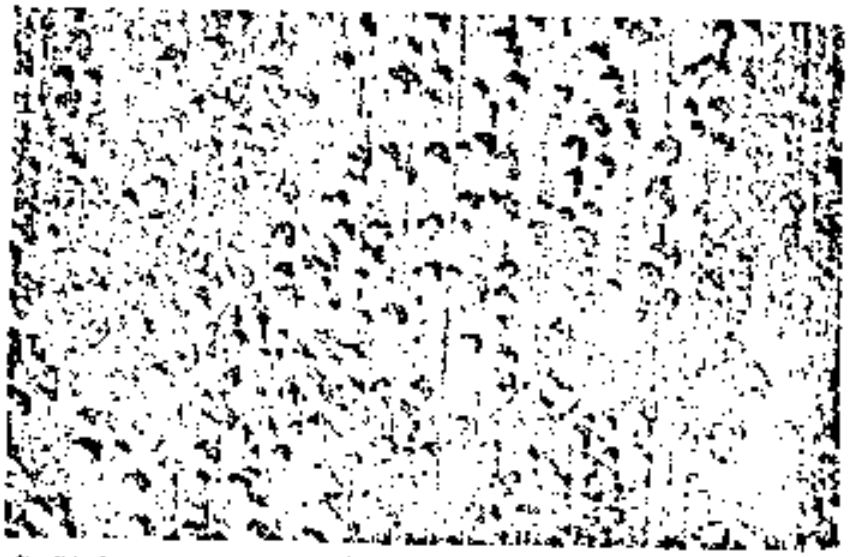

Abb. 5. Prüfblech aus Lat 1-Pb-Sn35 in Ölsäure, Luftzutritt an eingeritzter Stelle. Vergr. 1:1

Einfluß des Peroxidsauerstoffs

In je 100 ml Sojaöl wurden durch Einleiten von Sauerstoff bei erhöhter Temperatur Peroxide erzeugt. Nach Erreichen von Peroxidzahlen von etwa 50 bis 100 (nach Wheeler) wurden Prüfbleche (2 × 6 cm) eingehängt und die weitere Oxydation durch Einleiten von Stickstoff verhindert.

Tab. 10 zeigt klar die Abhängigkeit der Bleizunahme eines Öles von dessen Peroxidgehalt.

Die Entstehung von Peroxiden könnte durch Abfüllung unter Vakuum oder Schutzgas verhindert werden, wodurch nicht nur die Schwermetallaufnahme verringert, sondern auch die Haltbarkeit erhöht werden könnte, doch dürfte dies nur in spezielle Fälle sinnvoll sein.

Tabelle 10. *Einfluß des Peroxidgehaltes eines Öles auf die Pb-Aufnahme bei der Lagerung*

1 Peroxidzahl nach Wheeler zu Beginn	2 nach 1 Monat	3 Säurezahl 4 zu Beginn	5 nach 1 Monat	6 mg Blei in 100 ml nach 1 Monat
5	10	0,5	0,5	0,5
5	8	0,5	0,5	0,5
48	84	2,8	2,8	2,8
48	80	2,8	3,0	3,0
104	106	5,0	5,0	4,0
104	104	5,0	5,0	4,5

Einfluß der Weißblechoberfläche

Ergänzend sei auf die Möglichkeit hingewiesen, daß bei der Herstellung der Dosen Reaktionen des Lotmetalls erfolgen, z. B. Oxide oder Umsetzungsprodukte mit den Oberflächenbehandlungsmitteln des Weißblechs oder mit Lothilfsmitteln.

In der Oberfläche von frühegendem Lot in 375-ml-Fußbodendosen und 400-g-Fleischdosen konnten jedoch durchschnittlich nur 6 µg Blei in leicht abziehbarer Form (Abreiben mit alkoholsäurehaltiger Watte) nachgewiesen werden.

Diffusionsvorgänge

Man könnte annehmen, daß die beim Dosenangriff nach der Sterilisation gebildeten Metallseifen am Ort des Angriffs — also im Lot und an der Verzinnung — lokalisiert bleiben. Die Befunde widersprechen jedoch dieser Annahme, als z. B. auch in festen Fetten (Schmalzfleisch) in den vom Ort des Angriffs weiter entfernt liegenden Teilen Blei gefunden wird. Hieraus ist zu schließen, daß die Metallseifen trotz geringer Löslichkeit in fettartige Füllgüter diffundieren.

Dies ließ sich durch einen Modellversuch mit Schweineschmalz bestätigen.

Der Rumpf von Weißblechdosen wurde innen mit Lot 1, $PbSn_2$ überzogen. Die Dosen wurden mit Schweineschmalz gefüllt. Nach ein- und zweimonatiger Lagerung bei 25° C wurde der Bleigehalt in konzentrischen Schichten von je 1 - 2 mm Dicke bestimmt.

Der Diffusionskoeffizient D lag einheitlich bei 1 bis $4 \cdot 10^{-5}$, die Diffusion der Bleiseifen folgte somit dem Ausgleichsgesetz von Fick [23].

Potential von Blei, Zinn und Bleiloten

Aus dem Potentialverlauf von Blei, Zinn und zwei Bleiloten (L. $PbSn_{35}$ und L. $PbSn_2$) in Schmalzfleisch und Leberwurst, gegen die gesättigte Kalomelelektrode gemessen, ist zu ersehen (Abb. 6 und 7).

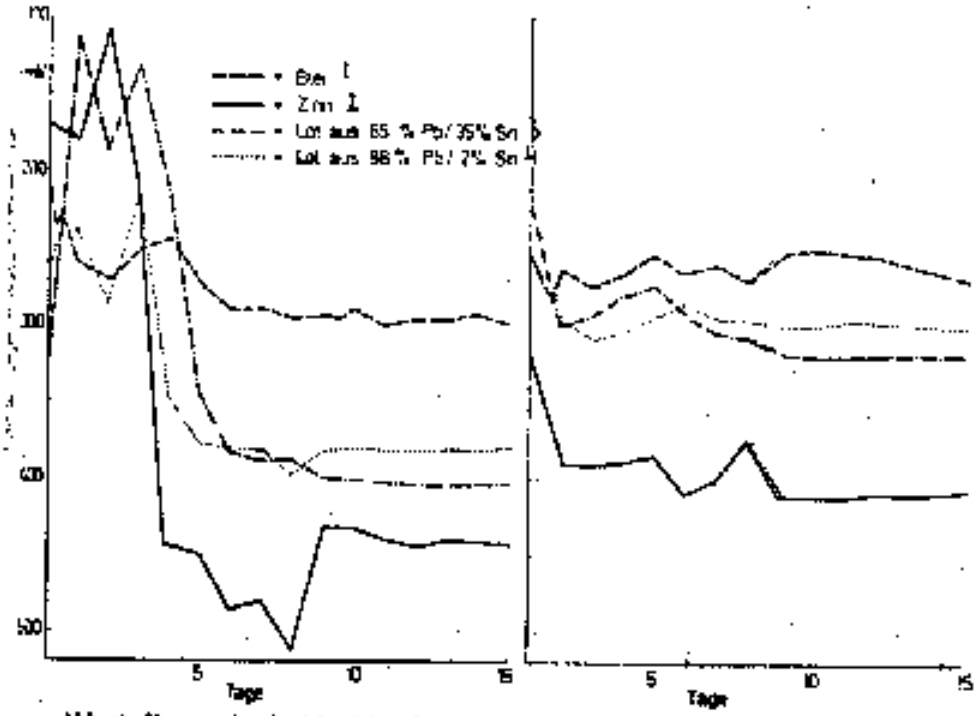

Abb. 6. Potentialverlauf in Schmalzfleisch

Abb. 7. Potentialverlauf in Leberwurst

1. Zinn weist nach einigen Tagen das niedrigste Potential auf und ist unedler geworden. Gegenüber Blei und den Bleiloten wird Zinn damit in Schmalzfleisch-Leberwurst ebenso, wie in den meisten wasserreichen Lebensmitteln, rasch zur Anode.

2. Das Potential von Blei liegt anfangs unter dem des Zinns und bleibt ungefähr auf dem nach einem Tag erreichten Wert stehen. Blei wird daher in kurzer Zeit edler als Zinn.

3. Die Potentiale der Lote L-PbSn 35 und L-PbSn2 liegen nahe beieinander stets zwischen den Potentialen von reinem Blei und reinem Zinn.

Aus dem Potentialverlauf der Doppelelektroden ist zu folgern, daß sich Zinn- und Bleiloten in den fetthaltigen Füllgütern Schmalzfleisch und Leberwurst ähnlich wie in wäßrigem Medium (BERGNER u. MEERMANN [1]). Dies bedeutet, daß diese Füllgüter anfänglich wohl Blei und Zinn in gleichem Maße angreifen, später wesentlich jedoch nur noch Zinn, solange etwa gleiche Flächen Blei und Zinn vorliegen. In der Praxis wird in blanken Dosen Bleilot mit kleiner Oberfläche als Kathode einer großen Zinnanode gegenüberstehen. In der lackierten Dose liegen die Verhältnisse anders, da ja entweder nur eine oder wenige kleine Oberflächen nach innen ringsum das Bleilot mit dem Füllgut in Kontakt kommt. Auch bleiben in der Praxis die angenommenen Mengen von Blei und Zinn auch im ungünstigsten Fall immer gering.

Bestimmung von Mikrogramm-Mengen Blei und Zinn in Fetten und fetthaltigem Material

I. Aufarbeitung des Probematerials

Zur Aufarbeitung von Fett und fetthaltigem Material zur Bestimmung von Schwermetallspuren stehen insbesondere die nasse und die trockene Veraschung zur Verfügung.

Die nasse Veraschung mit Schwefelsäure, Salpetersäure oder Perchlorsäure ist jedoch bei größeren Mengen Fett schwierig und auch langwierig. Außerdem werden mit den verwendeten Oxidationsmitteln stets große Metallmengen eingeschleppt.

Die trockene Veraschung ist die am häufigsten beschriebene und untersuchte Methode. Deshalb wurde auch auf diese Aufarbeitungsmöglichkeit das Hauptaugenmerk gerichtet.

Nach ASSOS und LIPSCOMB [24] ist eine entsprechende Vorsichtsmaßregel bei einer Bestimmung in aschearmem Material bei Veraschungstemperaturen um 500° C mit einem Verlust von 20% zu rechnen, während bei Gegenwart von Schwefelsäure bei dieser Veraschungstemperatur Blei in guter Ausbeute in der „Sulfatasche" gefunden wird. Durch Zusatz von Magnesiumnitratlösung oder Magnesiumacetatlösung kann die Veraschung beschleunigt werden.

Zinnverbindungen sind bei Temperaturen um 500° C kaum flüchtig. Nach Modifizierung von HORNCZYK u. MITACH [25] beschriebenen Verfahrens wurde wie folgt gearbeitet:

Reagenzien

(1) Schwefelsäure konz. pro anal.;
(2) Schwefelsäure, ultrarein;
(3) Magnesiumnitrat, 15%ige Lösung in 96%igem Äthanol.

Arbeitsvorschrift

Zu je 100 g Probe (Öl, Schmalzfleisch usw.) werden in einer Quarzgroßschale 100 g Schwefelsäure (1) zugesetzt. Auf dem Sandbad wird 8–10 Std lang auf ca. 300° C erhitzt.

Anschließend wird die Quarzschale auf dem Asbestdrahtnetz mit untergelegtem Tondreieck langsam weiter erhitzt, bis die Probe vollständig verkohlt ist. Nach Zusatzen von 1 ml Magnesiumnitratlösung (3) wird bei 400° C im Muffelofen während 5–8 Std weitergeglüht.

Selbst aus der Asche Bleispuren bestimmt werden, muß zur Veraschung Schwefelsäure ultrarein (2) verwendet werden.

Extraktion von Blei aus Speiseöl

Auf die Möglichkeit der Extraktion von Schwermetallspuren aus fettem Öl wurde wiederholt hingewiesen.

So erhitzen VIZNER und GRIMMER [26] das in Petroläther gelöste Fett mit Essigsäure, trennen nachher das als Acetat vorliegende Blei mit Alkohol ab.

HANSEN [27] extrahiert mit Schwefelsäure und mit einem Gemisch aus Salzsäure, Äther und Wasser.

TÄUFEL u. BAUMEL (28) verwenden zur Extraktion Trichloressigsäure. Mit Erfolg wurde ab die Extraktion mit Salpetersäure versucht, insbesondere weil Salpetersäure „dithizon-in" s Handel erhältlich ist.

Reagentien

(1) Salpetersäure, mit Dithizon geprüft:
(1a) Salpetersäure 1 + 3, (ca. 10%ig);
(3) Schwefelsäure, ultrarein.

Arbeitsvorschrift

100 bis 500 ml Öl werden in einer dithizongereinigten Polyäthylendusche mit 100 ml Salpetersäure (1a) versetzt und über Nacht auf einer Schüttelmaschine kräftig geschüttelt.

Nach dem Absitzenlassen und Abtrennen der Salpetersäure im Schütteltrichter wird noch einmal mit 50 ml bidestilliertem Wasser von Hand geschüttelt.

Die salpetersauren Auszüge sammelt man in einem 500 ml-Kjeldahl-Kolben, versetzt mit 5 ml Schwefelsäure, ultrarein (3) und erhitzt solange, bis die Salpetersäure abdestilliert und die Schwefelsäure farblos ist. Nach dem Erkalten füllt man 10 ml bidestilliertes Wasser zu, dampft ab und wiederholt diesen Vorgang noch einmal, um die Salpetersäure restlos zu entfernen. Die abgekühlte schwefelsaure Lösung versetzt man mit ca. 100 ml bidestilliertem Wasser, stellt mit 14 n-Ammoniak auf pH 8,5 ein und extrahiert das Blei wie nachfolgend beschrieben mit Dithizonlösung.

II. Mikro-Bleibestimmung

Von den von IWANTSCHENKO (30) mitgeteilten Methoden der Bleibestimmung wurde die Spektralfotometrie der Mischfarbe aus Bleidithizonat und freiem überschüssigem Dithizon angewandt.

Reagentien, Reinigung der Reagentien und Durchführung der Bestimmung waren im wesentlichen die gleichen, wie von BOROVIK u. MARTINE (1) beschrieben. Zusätzlich werden benötigt: Ammoniumhydroxyäthylcitratlösung, 10%ig und Natriumcyanidlösung, 5%ig.

Die Asche wird zunächst mit 5 ml Brom-Bromwasserstoffsäure-Gemisch einmal abgeraucht. Der verbleibende Rückstand wird mit 25 ml Salzlösung unter Bedecken mit einem Uhrglas eine Stunde auf dem Sandbad gekocht, um Calciumsulfat in Calciumcarbonat umzuwandeln. Nach dem Erkalten wird vorsichtig 6 n-Salzsäure bis zur sauren Reaktion zugesetzt, dann werden 10 ml Ammoniumhydroxyäthylcitratlösung zugefügt, um ein Ausfallen von Magnesium- oder Erdalkaliphosphaten zu vermeiden. Nach Neutralisation mit 14 n Ammoniak wird wie in der angegebenen Literatur weiter verfahren.

Der mittlere Blindwert aller zur Aufarbeitung verwendeten, gereinigten Reagentien betrug 1,5 µg Blei. Die Standardabweichung s betrug 0,3162 mit einer Varianz von 9,5%. Bei einer statistischen Sicherheit von 99,7% lag die Nachweisgrenze bei 2,2 µg Blei/25 ml*.

In einem Konzentrationsbereich von 2 bis 8 µg Blei/25 ml betrug der mittlere Fehler 20%.

Wurde Blei aus Öl mit Salpetersäure extrahiert und anschließend nach dem geschilderten Silikonverfahren bestimmt, so betrug der mittlere Blindwert der Reagentien 0,5 µg Blei. Bei einer Standardabweichung des Blindwertes von s = 0,075 betrug die Varianz der Werte 15,2%. Bei einer statistischen Sicherheit von 99,7% lag die Nachweisgrenze bei 1,5 µg Blei/25 ml*.

III. Mikro-Zinnbestimmung

Zur Bestimmung von Mikrogramm-Mengen Zinn kommt nach KORTÜM u. KORTÜM-DRONE (20) allein Dithiol das empfindlichere und wenigstens annähernd empfindliche Phenylfluoron mit einer Nachweisgrenze von etwa 0,2 µg Zinn/ml* in Frage, wobei allerdings die Nachweisgrenze hier stark von pH-Wert der photometrierten Lösung abhängt. Mit der Bestimmung von Mikrogramm-Mengen Zinn haben sich insbesondere DESMAZIÈRES (31) u. LEGER (32) beschäftigt. Unter Berücksichtigung dieser Arbeiten wurden die Zinnbestimmungen nach folgender Arbeitsvorschrift ausgeführt:

Reagentien

(1) Phenylfluoronlösung: 30 mg Phenylfluoron werden in wenig Methanol gelöst, in einem 50 ml-Meßkolben überspült, mit 4 ml HCl (2) versetzt und mit Methanol bis zur Marke aufgefüllt;
(2) Salzsäure 36%ig;
(3) Hydroxylaminsulfatlösung 10%ig in bidestilliertem Wasser;
(4) Methanol pro anal.;
(5) Bidestilliertes Wasser;
(6) Schwefelsäure, konz. mind. 98%ig, pro anal.;
(7) Salzsäure (6 n), in einer Pyrex-Destillationsapparatur destilliert;

* Messung in Lea-Kjivotte.

(8) Essigsäureäthylester pro anal.;
(9) L-Ascorbinsäure pro anal.;
(10) Kaliumferricyanidlösung, 10% ig in bidest. Wasser;
(11) Wasserstoffperoxid, 30%ig;
(12) Natriumacetatmatlösung, 10%ig, wäßrig.

Zur Zinnbestimmung wird das Material, wie im Abschnitt 1, 1 beschrieben, mit Schwefelsäure pro anal. versetzt.

Zur Umwandlung des bei der Veraschung von calciumreichem Material entstandenen Calciumsulfates kocht man die weniger konzentrierte Asche mit 25 ml Natronlauge (12) auf dem Sandbad 1 Std. lang. Der Rückstand wird mit Salzsäure (2) angesäuert und mit 20 ml bidestilliertem Wasser in einen 250 ml-Schüttelzylinder übergespült. Dann fügt man 5 ml Kaliumferricyanidlösung (10), eine Spatelspitze Ascorbinsäure (9) zu, wodurch die leicht rot gefärbte Lösung in 1 bis 2 min unter gelegentlichem Umschwenken eine schwach rosa Farbe erhält. Nach Zugabe von 20 ml Essigsäureäthylester (8) schüttelt man 3 min lang kräftig, läßt die wäßrige Phase in einen Erlenmeyerkolben und den leicht rötlich gefärbten Ester in eine 100 ml-Abdampfschale über.

Das Ausschütteln mit je 20 ml Ester wird noch zweimal wiederholt. Hierauf werden die vereinigten Extrakte auf einem leicht siedenden Wasserbad zur Trockne eingedampft. Der Rückstand wird mit 1 ml konz. Schwefelsäure (6) aufgenommen und nach Zugabe von 20 Tropfen Wasserstoffperoxid mit einem Uhrglas bedeckt 30 min lang in einem Trockenschrank von 150° gestellt. Dann gibt man 1 ml Hydroxylammoniumchloridlösung (3) zu und läßt die Schale weitere 30 min im Trockenschrank.

Nach Abspülen des Uhrglases und Überspülen mit Methanol in einen 25 ml Meßkolben gibt man 5 ml Phenylthiocarbazonlösung (1) zu und füllt mit Methanol bis zur Marke auf.

Nach 2stündigem Stehen im Dunkeln mißt man am Elko in der 1 cm-Küvette, Filter S, gegen bidestilliertes Wasser.

Die zur Anfärbung verwendete Phenylthiocarbazonlösung ist zwar tagelang haltbar; trotzdem empfiehlt es sich, bei jeder Messung den Blindwert zu bestimmen.

Konzentrationsbereich

Bis zu einer Konzentration von 11 µg/25 ml liegen die Eichpunkte auf einer Geraden, d. h. die Meßwerte weichen etwas ab. Die Messung im Konzentrationsbereich von 2 bis 11 µg/25 ml empfehlenswert.

Bestimmung des Verfahrens

Der mittlere Blindwert aller zur Aufarbeitung verwendeten Reagenzien betrug 0,50 µg/25 ml. Die Standardabweichung s betrug 0,0310 mit einer Varianz von 14,0%. Die Nachweisgrenze bei 1 µg Zinn/25 ml (1 cm-Küvette).

In einem Konzentrationsbereich von 2 bis 11 µg/25 ml betrug der mittlere Fehler 1%.

Zusammenfassung

Die Blei- und Zinnaufnahme der untersuchten Öle und fettreichen Lebensmittel in handelsüblichen Weißblechkonservendosen betrug unter den im folgenden angegebenen Lagerbedingungen weniger als 1 mg/kg bei z. T. erheblicher Streuung zwischen den Einzelbüchsen. Die gesundheitlich duldbaren Höchstmengen für Blei und Zinn wurden somit nicht überschritten.

Raffiniertes Sojaöl nahm in *lackierten, geflitzten Fülldeckeldosen* in 12 Monaten im Mittel 20 µg Pb/kg auf. Die Zinnaufnahme betrug in der gleichen Zeit etwa 10 µg/kg. Ein erhöhter Bleigehalt im Öl — teilweise über 100 µg/kg — war in gestanzten Dosen festzustellen, bei denen die Lotverbindung zwischen Kempf und Stülpdeckel aufgerissen war.

In Schweinefleisch und Rindfleisch aus *lackierten Falzdosen* waren nur 10 bis 20 µg/kg Pb und 25 bis 35 µg/kg Sn zu finden.

Eine etwas größere Blei- und Zinnaufnahme zeigten Schmalzfleisch und Leberwurst in *lackierten, überlappt gelöteten Dosen* (100 bis 70 µg Pb/kg, 40 bis 50 µg Sn/kg).

In *ungelackten Flachdosen* mit Muroldeckel ist offensichtlich der Kontakt mit dem Lot besonders stark und deshalb der Blei- und Zinngehalt der darin verpackten Leberwurstartigen Pasteten am größten (200 bis 800 µg Pb/kg, 20 bis 60 µg Sn/kg).

Wie Modellversuche zeigten, werden bei der Bleiaufnahme von Fett und fettreichen Füllgütern vorwiegend fettsaure Salze von Blei gebildet. Der Angriff wird durch steigenden Säure- und Peroxidgehalt größer. Zinn und Lot L-PbSn35 wurden stärker angegriffen als Blei und Lot L-PbSn2. Die Metallseifen bleiben nicht am Ort der Entstehung, sondern diffundieren in das Füllgut. Es konnte gezeigt werden, daß diese Diffusion dem Ausgleichsgesetz von Fick folgt.

Vorschriften zur Aufarbeitung des Probenmaterials (Sulfatasche oder Salpetersäureextraktion) und zur Bestimmung von Blei (Dithizon-Methode) und von Zinn (Phenylfluoron-Methode) werden angegeben. Die Nachweisgrenze für Pb liegt je nach Aufarbeitungsmethode bei 1,5 bis 2,2 µg/25 ml bei Messung in 1 cm-Küvette, Streuungsbereich 20%. Die Nachweisgrenze für Sn^{2+} liegt bei 1 µg/25 ml bei Messung in 5 cm-Küvette, Streuungsbereich 5%.

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Original Communications

Fecal Excretion of Iron and Tin by Men Fed Stored Canned Foods

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In foods stored in tinplate containers there may be solution of metal with transfer of tin and iron into the food. The degree of transfer depends on many factors such as the type of product, time and temperature of storage, and whether the containers were plain or enameled. Based largely on animal studies, and the fact that canned foods are regularly eaten with impunity, it is generally agreed that at the levels found in conventional canned products, the dissolved tin and iron are essentially innocuous.[1-6]

Considering the duration and extent to which metal containers are used, it is surprising that the most extensive balance study of dietary tin to be found in the literature consisted of a twenty-eight-day study of one subject given an uncontrolled but measured diet, in whom fecal output slightly exceeded intake.[1] There have been no human balance studies concerned specifically with utilization of iron gained from cans.

A unique opportunity for the study in man of absorption of adventitious tin and iron was presented coincidental to a feeding experiment conducted at the U. S. Army Medical Research and Nutrition Laboratory for the purpose of assessing the adequacy of pyridoxine level in stored rations.[7] The data here presented demonstrate significant absorption of iron but not of tin from corroded containers.

METHODS AND MATERIALS

Each carton of the individual combat rations (C ration) used contained six individually packed one-day menus, assembled from a single procurement of each component. A list of the canned components is given in Table 1. Alternate cartons were placed in controlled storage in our laboratories at 1°C. (Cr) and 37°C. (Cu) and held for twenty months prior to study. These storage temperatures and times departed widely from usual practise and were chosen to emphasize and accelerate storage effects. (That these conditions would rarely be encountered in normal food handling practise was shown by the fact that some of the cans examined for chemical

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TABLE I
Tin and Iron Content* of C Ration Components Stored for Twenty Months at the Indicated Temperatures

Component	Menu No.	Iron		Tin	
		1°c.	37°c.	1°c.	37°c.
Packed in 211 by 304 cans 8 oz. net weight					
Plain tinplate					
Apricots	6	3	6	32	414
Cherries, white	4	6	32	48	470
Fruit cocktail	3	3	10	48	427
Peaches	1, 5	4	8	28	254
Pears	2	5	103	22	538
Packed in 300 by 308 cans 11.6 to 12 oz. net weight					
Plain tinplate					
Beans w/franks	1, 3	18	22	48	262
Beans w/pork	2	17	13	30	144
Beef stew	4	15	15	22	84
Chop suey	6	15	18	27	100
Hamburger	5, 6	34	34	15	43
Meat w/beans	4	24	19	46	302
Meat w/spaghetti	1	15	20	38	288
Enameled tinplate					
Beef w/peas	6	28	130	NF	NF
Chicken w/noodles	1, 6	11	14	NF	Trace
Chicken w/vegetables	2, 3	24	17	NF	12
Ham and potatoes	3	10	40	NF	8
Meat w/noodles	5	15	22	NF	NF
Pork sausage patties	2, 4	24	71	NF	NF

* mg. per kg. of wet food (p.p.m.)
NF = none found.

analysis were swollen from gases evolved during reaction between the food and the metal.) A control diet (A) composed of a single menu of fresh foods was formulated to approximate the composition of the packaged ration.

Nine young adult male volunteer subjects received each diet for twenty-four days (four cycles of the six C ration menus), the order of presentation following a Latin square design. Details of subjects and management have been presented elsewhere.[7]

The proximate composition,[8] tin[9] and iron[9] content of the diets were determined from three to six composite samples each of which was a blend of two daily food allowances. Quantitatively collected feces were analyzed for moisture,[8] nitrogen,[8] tin and iron content; and urine specimens, for nitrogen and tin content. In addition to the diets, tin and iron levels of three-can composites of individual items from the same lot of stored C rations were measured. One sample of solids derived from 6 L. of tap water was analyzed for tin and iron content; no tin was found and the iron content of 0.1 μg. per ml. was sufficiently low to be disregarded in the calculation of intakes.

RESULTS

Component analyses, presented in Table I, revealed tin levels above 50 p.p.m. in all items packed in plain tin-plate cans and stored at 37°c., with the exception of hamburgers which had no liquid phase. All the fruits and three meat components contained more than 200 p.p.m. of tin. On the other hand, items packed in plain tin-plate cans and held frozen (1°c.) contained less than 50 p.p.m. Iron content of most fruits also increased as a result of storage at high temperature (37°c.) In contrast, in mixed meat items there was no appreciable gain in iron with the exception of a few products packed in enameled cans in which pitting due

TABLE II

Iron and Tin Content* of Diets Stored for Twenty Months at the Indicated Temperatures

Diet	Dry Solids (gm./day)	Iron		Tin	
		1°c.	37°c.	1°c.	37°c.
Control (A)	722	31† (30–32)	...	13 (11–15)	...
C Ration (C)					
1	786	30 (23–35)	44 (40–51)	52 (46–56)	310 (273–356)
2	778	31 (28–42)	143 (47–235)	28 (23–30)	247 (210–304)
3	824	28 (23–31)	70 (52–113)	35 (32–40)	232 (172–311)
4	782	35 (33–39)	135 (45–300)	43 (36–54)	201 (25–299)
5	846	34 (30–37)	37 (31–43)	25 (21–33)	136 (116–162)
6	778	42 (32–53)	54 (33–65)	16 (Trace–23)	90 (72–125)
			mg. per day		
A‡	723	22.1 (21.5–23.0)	...	9.5 (8.6–10.8)	...
C‡	800	26.7 (22.3–31.0)	64.4 (23.8–105.0)	20.3 (20.0–31.4)	102.8 (115.8–205.7)

* mg. per kg. dry solids (p.p.m.). The control diet was not stored beyond conventional refrigeration of fresh products.

† Mean of three to six samples, and range in parentheses.

‡ These values are given in mg. per day. The figures for C represent the average of all C ration menus.

to failure of the enamel permitted attack on the base metal.

Table II lists the tin and iron content of composite menus of the rations. The variation in content of any given menu of stored canned rations was high and typical of normally encountered can to can variation. Average tin content of the fresh diet was 13 p.p.m. on a dry solids basis; of the C ration stored at 1°c., 33 p.p.m.; and of the rations stored at 37°c., 204 p.p.m., a sixfold increase. Iron content of the A and C1 rations was similar, 30 p.p.m. on a dry solids basis, and that of the C11 rations more than double that, averaging 80 p.p.m. Average daily intakes of tin were 10 mg. from the control diet, 26 mg. from C1 and 103 mg. from C11. Iron intakes were 22, 27 and 64 mg., respectively.

Fecal iron content was 18 mg. per day during the A diet period, indicating retention of approximately 4 mg., and values were comparable during the twenty-four days of feeding the C1 ration (21 mg. ingested and 6 mg. retained, Table III). When the ration stored at high temperature (37°c.) was given, only 35 mg. of the average 64 mg. intake appeared in the feces. Thus apparent daily retention of iron from the C11 ration was 29 mg.

During the A and C1 diet periods, fecal excretion of tin was slightly greater than the estimated intake and during the C11 diet period, slightly less. In all cases, however, individual excretion values were equally dispersed on both sides of the estimated mean intake and the range of observed fecal values was probably within the possible range of intakes based on menu variance. Data on urinary excretion of tin are not shown because only trace amounts were detected and these were detected uniformly in all collection periods.

All subjects were in positive nitrogen balance during all periods of study (Table IV), and no difference was attributable to dietary treatments. The protein of C ration was less digest-

TABLE III
Fecal Iron and Tin Content

Subject	Order of Feeding*			Iron (mg./day)			Tin (mg./day)		
	A	Cr	Crr	A	Cr	Crr	A	Cr	Crr
A	1	3	3	16.6	20.7	57.5	10.8	27.0	100.7
B	3	3	1	17.5	22.2	13.4	9.7	30.0	137.0
C	3	1	2	19.7	21.0	39.3	11.9	34.8	150.1
D	1	3	2	17.1	22.1	20.6	12.3	31.1	148.5
E	3	3	1	18.6	20.3	19.7	9.4	29.6	164.9
F	3	2	1	18.4	23.8	35.4	10.9	33.8	109.3
G	1	2	3	18.2	20.5	48.2	9.9	27.2	180.8
H	2	1	3	19.8	20.7	38.3	12.0	32.1	153.1
I	3	1	2	15.9	18.6	33.1	9.6	35.5	169.6
Average fecal excretion				18.0	21.1	34.9	10.7	31.3	158.0
Average dietary intake				23.1	20.7	64.4	9.5	30.3	162.8

* Order in which diets A (fresh control), Cr (ration stored at 1°c.) and Crr (ration stored at 37°c.) were administered for twenty-four day feeding periods.

ible than that of the fresh diet and poorer in the ration stored at high temperature (37°c.) than in that stored in the cold (1°c.). Respective percentages of apparent digestibility were 91, 84 and 81, for the A, Cr and Crr diets.

COMMENTS

Fecal excretion of iron accounted for 82 per cent of that ingested in the control diet and 78 per cent of that in the processed ration held in cold storage (1°c.). Thus, apparent absorption was 18 to 22 per cent of dietary iron, or 4 to 6 mg. per day. These values fall within the high normal range.

During consumption of C ration stored at 37°c., fecal iron output was only 54 per cent of the average intake of 64 mg. per day; 30 mg. was absorbed daily, or 700 mg. for the total twenty-four-day period. This indicates that of the additional 38 mg. of iron contributed by the containers, roughly 66 per cent was absorbed. These data support the observation of

TABLE IV
Nitrogen Balance and Protein Digestibility

Subject	A*				Cr*				Crr*			
	Nitrogen (gm./day)			Protein Digestibility† (%)	Nitrogen (gm./day)			Protein Digestibility† (%)	Nitrogen (gm./day)			Protein Digestibility† (%)
	Fecal	Urinary	Balance		Fecal	Urinary	Balance		Fecal	Urinary	Balance	
A	1.86	17.93	1.94	91.1	3.73	18.90	2.00	81.5	4.47	17.85	1.80	81.5
B	2.08	14.58	4.43	90.1	3.80	15.90	4.42	81.2	3.90	14.69	5.44	83.5
C	2.13	15.50	3.41	89.0	4.12	16.79	3.18	82.0	5.21	15.70	3.18	78.3
D	2.07	15.61	3.86	90.2	4.27	14.80	2.04	82.3	4.22	13.26	3.24	78.1
E	2.00	15.81	3.73	90.5	3.25	17.93	3.58	83.0	4.00	17.25	2.87	83.1
F	1.80	16.43	2.32	91.7	3.88	16.46	3.78	83.9	4.41	16.96	2.73	81.7
G	1.91	15.83	3.32	90.9	4.08	16.67	3.13	83.0	4.97	16.60	2.53	70.4
H	1.82	15.45	2.82	91.4	3.63	17.50	2.97	84.9	4.37	16.16	4.38	81.1
I	2.03	14.12	4.62	90.1	3.63	15.07	4.55	84.9	3.82	16.42	3.87	84.2
Average	1.98	15.81	3.48	90.6	3.89	16.88	3.46	84.1	4.52	16.24	3.35	81.3

* Intake of nitrogen from control diet A = 21.07 (20.67–21.20) gm. per day; from C ration stored at 1°c. (Cr) or 37°c. (Crr) = 24.11 (23.33–24.98) gm. per day.
† Apparent, i.e., not corrected for metabolic nitrogen loss.

Theriault and Fellers[8] that iron gained from cans was nearly completely available to the rat. Bothwell et al.[9] reported that Bantu subjects excreted 60 to 90 per cent of adventitious iron present in a test dose of beer brewed in iron containers according to native custom, but absorption matched that of ferric salts in these subjects habituated to high iron intake and in whom hemosiderosis is common.

This study does not resolve the question of why absorption of iron from the C11 ration was so high in the face of presumably low physiological needs, but it may be cogent to refer to the previously reported observation by Harding et al.[5] that the C11 ration was marginal with respect to pyridoxine as indicated by the increased excretion of xanthurenic acid following the administration of tryptophan. (The A, C1 and C11 diets provided 4.28, 2.76 and 1.93 mg. of pyridoxine per day.) Hyperferremia is found in pyridoxine-responsive anemia[10,11] and, at high dosages of both, pyridoxine was shown to reduce iron absorption in pregnant women.[12] Increased iron absorption has been reported in pyridoxine-deficient swine,[13] and in deficient rats when the diet provided 10 mg. per cent of iron but not when smaller doses were given.[14] The C11 ration contained a comparably high level of available iron, 8 mg. per cent.

Earlier studies on tin absorption and excretion have been reviewed elsewhere.[1-3] Our data conform, demonstrating that tin from canned foods is either not appreciably absorbed or is very promptly excreted into the bowel. Since nitrogen absorption from the C11 ration was somewhat lower than that of the C1 ration, it is possible that the tin in the food was bound to protein in an indigestible complex as suggested by Goss.[15] However, in our study tin effects are confounded with temperature effects so this question must be resolved in more definitive studies.

SUMMARY

Packaged military rations (C ration) were stored at 1° and 37°c. for twenty months prior to feeding to human subjects for successive twenty-four-day periods. Storage for an excessive time at the unusually elevated temperature increased the iron and tin content of the ration from 30 to 50 and 200 p.p.m. (dry solids), respectively. Fecal excretion accounted for all the tin ingested, and for about 80 per cent of the iron from a control diet and the ration stored at cold temperatures (1°c.) but only for 54 per cent of the 64 mg. daily intake of iron provided by the ration stored at 37°c. A relationship of iron absorption to marginal pyridoxine intake is suggested.

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PHARMOCODYNAMICS--ON THE TOXICITY AND MEDICAL DOSE OF STANNOUS CHLORIDE FOR THE GUINEA PIG, THE RABBIT, AND THE MOUSE

Notes of Robert Deschiens, Didier Bertrand, and Robert Romand, presented by Gabriel Bertrand

Stannous oxide and Stannous chloride possess anthelmintic properties. In the guinea pig, the rabbit, and the mouse, lethal doses are from 40 to 60 mg per kilogram per day for 6 days, and medical doses are from 4 mg per kilogram per day for 8 consecutive days; the chemotherapeutic coefficient: 1/10 to 1/15.

It is known that tin and certain mineral salts, stannous oxide in particular, are used effectively in the treatment of certain human helminthiases (teniasis, bilharziasis intestinal).

Stannous oxide, insoluble in water when it is ingested, appears to be partially transformed by hydrochloric acid in the stomach into stannous chloride, a soluble salt, which, penetrating into the constitution of the host and of the parasite, can exert parasiticidal pharmocodynamic effects.

In intestinal bilharziasis from S. mansoni, the activity of stannous oxide administered per os[1,2,3] appears to be connected to the partial transformation of this salt into stannous chloride in the body with the absorption of the former by the mesenteric vessels and with its maximum concentration in the portal system where the schistosomes live.

In the course of pharmacodynamic research, which had for its purpose the substitution of ~~tin~~ stannous chloride in intramuscular injections for ~~tin~~ stannous oxide administered per os, we came to define the toxic doses (in slow impregnation) and therapeutic doses of ~~tin~~ stannous chloride for the guinea pig, the rabbit, and the mouse.

The documentation that we have on the toxicity of mineral salts of tin is limited. Administration per os to the dog of 4 to 6 g of ~~tin~~ stannous chloride (about 200 mg/kg) causes in the animal grave and occasionally fatal intestinal disorders; in intramuscular injections, this same dose causes convulsions, dyspnea, and fatal paralysis. In man, intoxication from ~~tin~~ stannous chloride (tin protochloride) causes gastrointestinal disorders with vomiting and the sensation of a metallic taste in the mouth.[4]

With regard to organic salts of tin, we know that tetraethyl tin is toxic for the dog (in intravenous injection in doses ranging from 0.025 to 1 mmol/kg of a 2-o/oo solution in two hours to three days.[5]

On the other hand, on the biochemical level, we know that tin is found normally in certain viscera of mammals and that in the ox it is localized electively in the mucous membrane of the tongue where its concentration may reach 2 to 4 mg/kg.[6] Furthermore, it has been established that tin marked ^{113}Sn after administration to the dog in the form of tetraethyl tin is localized electively on the lungs, the kidneys,

the salivary glands, the heart, the tongue, the skin, and the nervous system.[7]

In the quinea pig (4 animals of 300 to 500 g tested, by intramuscular injections), the lethal dose of ~~tin~~ stannous chloride administered for 6 consecutive days was established by us at 40 mg per kilogram per day or 20 mg of tin per kilogram and 240 mg per kilogram of ~~tin~~ stannous chloride in total. A well tolerated medical dose was on the order of 4 mg per kilogram of ~~tin~~ stannous chloride per day or 2 mg of tin per kilogram administered for 6 consecutive days. The chemotherapeutic coefficient is then 1/10.

In toxic doses, the lesions observed in the guinea pig reveal a congestion of small hemorrhagic foci in the lungs, a parenchymatous hepatitis, and an epithelial nephritis; clinically, one notes dyspnea, watering of the eyes, a generalized trembling, and a paralysis of the rear quarters. Examination for tin in the liver and in the kidneys by the spectrographic method is positive.

In the rabbit (4 animals of 2 kg tested, by intramuscular injections), the lethal and medical doses were the same as those noted for the quinea pig; the lesions and clinical signs were comparable.

In the mouse (8 animals of 20 g tested, by intramuscular injections), the lethal dose of ~~tin~~ stannous chloride was 60 mg per kilogram per day administered for 8 consecutive days or 30 mg of tin per kilogram per day and 360 mg per kilogram of ~~tin~~ stannous chloride in total. The anthelmintic medical dose for S. mansoni is 4 mg of ~~tin~~ stannous chloride per kilogram per day for 8

consecutive days. The chemotherapeutic coefficient is 1/15.

The intoxicated animals presented hepatic and renal changes (parenchymatous hepatitis, epithelial nephritis). In them as in those that received medical doses, we noted upon autopsy the presence in the liver of dark brown granular deposits with a metallic appearance, which the spectographic examination permitted us to recognize as being rich in tin.

FOOTNOTES

1. R. Deschiens, Bull. Soc. Path. Exotique, 47, 1954, p. 79.

2. J. Mauze and G. Arnaud, Bull. Soc. Path. Exotique, 47, 1954, p. 77.

3. J. Bellon, Bull. Soc. Path. Exotique, 48, 1955, p. 197.

4. R. Fabre, Lecons de Toxicologie, Paris, 2, 1945.

5. F. Gaujolle, M. Lesbre, A. Bru, D. Meynier, and Y. Bru, Comptes Rendus, 240, 1955, p. 1829.

6. G. Bertrand, Comptes Rendus, 192, 1931, p. 780.

7. F. Caujolle, M. Lesbre, A. Bru, D. Meynier, and Y. Bru, Comptes rendus, 240, 1955, p. 1829.

(1) M. Pierron, *Mém. du Muséum nat. d'Hist. nat.*, Nouv. sér., **21**, 1948, p. [illegible].

(2) L. J. Weiss, Bulletin, n° 208, *Commonw. coith. scientific industr. Res. Organization*, Australia, 1945, p. 33-34.

(3) M. Gressner, *Medizinalzeitung* [illegible] *Lande Pharmazeutin*, **7**, 1890, p. 46-47.

PHARMACODYNAMIE. — *Sur la toxicité et la dose médicamenteuse du chlorure stanneux pour le Cobaye, le Lapin et la Souris.* Note de MM. Roger Desmars, Didier Bertrand et Robert Rossard, présentée par M. Gabriel Bertrand.

L'oxyde stanneux et le chlorure stanneux présentent des propriétés antiparasitaires. Chez le Cobaye, le Lapin et chez la Souris les doses léthales sont de [illegible] à 60 mg par kilogramme et par jour, pendant 6 jours et les doses médicamenteuses de 4 mg par kilogramme et par jour, pendant 8 jours consécutifs; coefficient chimiothérapeutique : 1/10e à 1/15e.

On sait que l'étain et certains de ses sels minéraux, en particulier l'oxyde stanneux, sont utilisés avec efficacité dans le traitement de certaines helminthiases humaines (taeniasis, bilharziose intestinale).

L'oxyde stanneux, insoluble dans l'eau lorsqu'il est ingéré, semble partiellement transformé par l'acide chlorhydrique de l'estomac en chlorure stanneux, sel soluble, qui, pénétrant dans l'économie de l'hôte et du parasite, peut exercer des effets pharmacodynamiques parasiticides.

Dans la bilharziose intestinale à *S. mansoni*, l'activité de l'oxyde stanneux administré *per os* (1), (2), (3) paraît se relier à la transformation partielle de ce sel en chlorure stanneux dans l'économie, à l'absorption de celui-ci par les vaisseaux mésentériques et à sa concentration maxima dans le système porte où vivent les schistosomes.

Au cours de recherches pharmacodynamiques ayant pour but de substituer le chlorure stanneux, en injections intramusculaires, à l'oxyde stanneux, administré *per os*, nous avons été amenés à préciser les doses toxiques (en imprégnation lente) et thérapeutiques, du chlorure stanneux, pour le Cobaye, le Lapin et la Souris.

La documentation qu'on possède sur la toxicité des sels minéraux de l'étain est restreinte. L'administration *per os*, au Chien, de 4 à 6 g de chlorure stanneux (200 mg/kg environ) provoque, chez l'animal, des troubles intestinaux graves et parfois mortels; en injections intramusculaires, cette même dose détermine des convulsions, de la dyspnée et une paralysie mortelle. Chez l'Homme, l'intoxication par le chlorure stanneux (protochlorure d'étain) suscite des troubles gastro-intestinaux avec vomissements et sensation de saveur métallique dans la bouche (1).

En ce qui concerne les sels organiques de l'étain, on sait que le tétraéthylétain est toxique pour le Chien (en injection intraveineuse, à des

doses comprises entre 0,005 et 1 mmol kg d'une solution à [illegible] en 24 à trois jours (2).

D'autre part, sur le plan biochimique, on sait que l'étain se rencontre normalement dans certains viscères de Mammifères et que, chez le Bœuf, il se localise électivement dans la muqueuse de la langue où sa concentration peut atteindre 2 à 4 mg kg [illegible]. En outre, il a été établi que l'étain marqué ^{113}Sn, après administration au Chien sous la forme de tétraéthylétain, se localise électivement sur les poumons, le cœur, les glandes salivaires, le cœur, la langue, la peau et le système nerveux [illegible].

Chez le Cobaye (4 animaux de 300 à 500 g éprouvés, par injections intramusculaires), la dose letale du chlorure stanneux administré pendant 6 jours consécutifs, a été établie par nous à [illegible] mg par kilogramme et par jour, soit [illegible] mg d'étain par kilogramme et [illegible] mg par kilogramme de chlorure stanneux, au total. La dose médicamenteuse la [illegible] a été de 4 mg par kilogramme de chlorure stanneux et par jour, soit [illegible] mg d'étain par kilogramme, administrés pendant 6 jours consécutifs. Le coefficient chimiothérapeutique est donc de [illegible].

Aux doses toxiques, les lésions observées, chez le Cobaye, revêtent une congestion à petits foyers hémorragiques des poumons, une néphrite parenchymateuse et une néphrite épithéliale; cliniquement, on note de la dyspnée, du larmoiement, un tremblement généralisé et une paralysie du train postérieur. La recherche de l'étain dans le foie et dans le rein, par la méthode spectrographique se montre positive.

Chez le Lapin (4 animaux de [illegible] kg éprouvés, par injections intramusculaires), les doses léthale et médicamenteuse ont été les mêmes que celles notées chez le Cobaye; les lésions et les signes cliniques ont été comparables.

Chez la Souris (8 animaux de 20 g éprouvés, par injections intramusculaires), la dose léthale du chlorure stanneux a été de [illegible] mg par kilogramme et par jour, administrés pendant 8 jours consécutifs, soit [illegible] mg d'étain par kilogramme et par jour et [illegible] mg par kilogramme de chlorure stanneux, au total. La dose médicamenteuse antihelminthique pour *N. mansoni* est de 4 mg de chlorure stanneux par kilogramme et par jour, pendant 8 jours consécutifs. Le coefficient chimiothérapeutique est de 1,15.

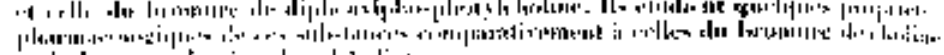
Les animaux intoxiqués ont présenté des altérations hépatiques et rénales : hépatite parenchymateuse, néphrite épithéliale. Chez eux, comme chez ceux ayant reçu des doses médicamenteuses, il a été noté, à l'autopsie, la présence dans le foie de dépôts grandeux bruns foncés, d'aspect métallique, que l'examen spectrographique a permis de reconnaître riches en étain.

(1) H. Bertrand, *Bull. Soc. Path. exotique*, 47, 1954, p. 79.
(2) J. Mazel et G. Arnaud, *Bull. Soc. Path. exotique*, 47, 1954, p. 77.
(3) J. Bouin, *Bull. Soc. Path. exotique*, 48, 1955, p. 197.
(4) G. Fabre, *Leçons de bactériologie*, Paris, 2, 1955.
(5) F. Chodat, M. Lesser, A. Bur, D. Messier et Y. Bur, *Comptes rendus*, 250, 1955, p. 1899.
(6) G. Bertrand, *Comptes rendus*, 192, 1931, p. 780.
(7) F. Chodat, M. Lesser, A. Bur, D. Messier et Y. Bur, *Comptes rendus*, 250, 1955, p. 1899.

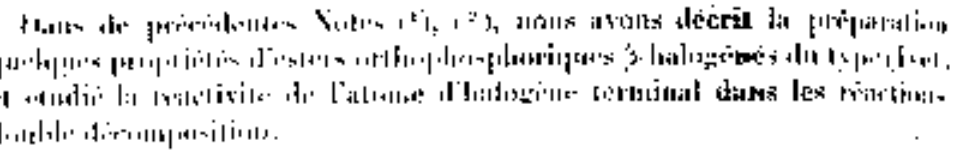
PHARMACODYNAMIE. — *Sur de nouveaux esters phosphoryles de la choline.* Note (1) de MM. René Hazard, Jean Cheymol, Pierre Chabrier et Mlle Annick Chayvas Gentil, transmise par M. Léon Binet.

Les auteurs décrivent la préparation du dibromure de phénylphosphorylbétaïne et celle du bromure de diphénylphosphorylcholine. Ils étudient quelques propriétés pharmacologiques de ces substances comparativement à celles du bromure de choline et du bromure de phosphorylcholine.

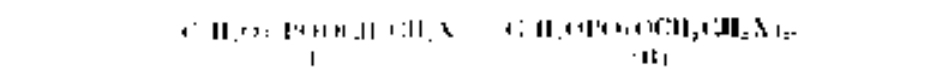
Dans de précédentes Notes (1), (2), nous avons décrit la préparation et quelques propriétés d'esters orthophosphoriques β-halogénés du type (I) et (II) et étudié la réactivité de l'atome d'halogène terminal dans les réactions de double décomposition.



Dans cette Note nous étudierons une réaction d'addition conduisant à de nouveaux dérivés de la choline (III) et (IV) dont nous indiquerons quelques propriétés pharmacologiques.



(III)



(IV)

Pour obtenir ces composés (III) et (IV) il suffit de mettre en contact l'ester phosphorique bromé (I) ou (II) et la triméthylamine en milieu benzénique.

Bromure de diphénylphosphoryl-bétaïne (III). — Dans un flacon bouché, contenant 25 ml de benzène on dissout 10,5 g d'ester β-bromoéthylique diphénylphosphorique et 18 ml d'une solution benzénique de triméthylamine à 30 % (poids/vol). On abandonne à température ordinaire. Un précipité cristallin se sépare peu à peu. Après 24 jours de contact on le recueille. Poids 8,5 g.

Rendement de l'opération : 70 % environ.

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DRAFT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date September 29, 1972

Laboratory No. 0901 t
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-33 (Stannous chloride)

Examination Requested: Teratologic evaluation of FDA 71-33 in mice.

Procedure: See Appendix I

Results: See Tables I through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 50 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the fourteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEAR LABORATORIES, INC.

Groups: 131 through 136

Material: FDA 71-33

Date September 29, 1972

Laboratory No. 0901 t

Table 1

Fate Summary
(Mice)

Group	Material	Dose ** mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
131	Sham	0.0	29	21	28	21
132	Aspirin*	150.0	24	20	24	20
133	FDA 71-33	0.5	26	23	26	23
134	FDA 71-33	2.3	25	23	25	23
135	FDA 71-33	11.0	22	20	22	20
136	FDA 71-33	50.0	25	21	25	21

* Positive Control
** Administered as a water solution; 1.0 ml per kg of body weight

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 131 through 136 | Laboratory No. 0901 t

Table 3

Material FDA 71-33 | Date September 29, 1972

Summary of Skeletal Findings*
(Mice)

Findings	Group No.	131	132	133	134	135	136
	Dose (mg/kg)	Sham	Aspirin**	0.5	2.3	11.0	50.0
Live Fetuses Examined (at term)		160/21	166/19	171/23	189/23	156/20	167/21
Sternebrae							
Incomplete oss.		67/19	113/19	105/21	121/23	95/19	115/20
Scrambled							
Bipartite		4/2	7/7	8/6	10/8	6/5	7/5
Fused							
Extra				1/1			
Missing		17/5	37/16	26/10	22/7	13/7	34/12
Other							
Ribs							
Incomplete oss.							
Fused/split							
Wavy							
Less than 12							
More than 13		7/6	8/7	11/8	13/8	11/7	8/5
Other							
Vertebrae							
Incomplete oss.		1/1		1/1			
Scrambled							
Fused							
Extra ctrs. oss.							
Scoliosis							
Tail defects							
Other							
Skull							
Incomplete closure							
Missing							
Craniostosis							
Other							
Extremities							
Incomplete oss.		8/2	3/2	2/2	1/1	1/1	
Missing							
Extra							
Miscellaneous							
Hyoid; missing		43/13	54/17	26/11	29/14	29/14	43/15
Hyoid; reduced		13/9	19/14	21/13	22/12	22/13	28/16

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte
** Positive control at 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 131 through 136 Date September 29, 1972

Material FDA 71-33 Laboratory No. 0901 t

Table 3-a
Summary of Soft Tissue Abnormalities
(Mice)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
131	Aspirin*	150.0	A 1407	1	Meningoencephalocele
134	FDA 71-33	2.3	T 1037	1	Meningoencephalocele
136	FDA 71-33	50.0	T 1098	1	Meningoencephalocele

* Positive control at 150.0 mg/kg

Group: 2 through 136 Date: September 29, 1972

Material: FDA 71-33 Laboratory No.: 0901 E

Tabl
Reproduction Data
(Mice)

Group:	131	132	133	134	135	136
Dose (mg/kg):	Sham	Aspirin**	0.5	2.3	11.0	50.0
Pregnancies						
Total No.	21	20	23	23	20	21
Died or Aborted (before Day 17)	1	0	0	0	0	0
To term (on Day 17)	21	20	23	23	20	21
Corpora Lutea						
Total No.	379	358	375	400	316	365
Average/dam mated	13.1	14.9	14.4	16.0	14.4	14.6
Live Litters						
Total No.*	21	19	23	23	20	21
Implant Sites						
Total No.	248	259	279	290	245	263
Average/dam*	11.8	13.0	12.1	12.6	12.3	12.5
Resorptions						
Total No.*	18	16	27	15	21	14
Dams with 1 or more sites resorbed	6	9	13	11	11	7
Dams with all sites resorbed	0	1	--	--	--	--
Per cent partial resorptions	28.6	45.0	56.5	47.8	55.0	33.3
Per cent complete resorptions	--	5.00	--	--	--	--
Live Fetuses						
Total No.	229	238	248	269	223	241
Average/dam*	10.9	11.9	10.8	11.7	11.2	11.5
Sex ratio (M/F)	1.08	0.93	1.19	1.13	0.87	0.96
Dead Fetuses						
Total No.*	1	5	4	6	1	8
Dams with 1 or more dead	1	5	4	5	1	7
Dams with all dead	0	--	--	--	--	--
Per cent partial dead	4.76	25.0	17.4	21.7	5.00	33.3
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.91	0.80	0.88	0.90	0.89	0.86

*Includes only those dams examined at term.
**Positive Control: 150.0 mg/kg

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FOOD AND DRUG RESEA[illegible] LABORATORIES, INC.

Groups 131 through 136

Species FDA 71-33

Date September 29, 1972

Laboratory No. 0901 t

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	Day 6	Day 11	Day 15	Day 17**
		mg/kg	g				
131	Sham	0.0	31.1	34.9	37.9	45.7	52.1 (21)
132	Aspirin***	150.0	33.1	36.5	38.5	47.2	53.0 (20)
133	FDA 71-33	0.5	30.5	33.3	36.8	44.5	50.0 (23)
134	FDA 71-33	2.3	32.3	35.2	39.1	47.2	52.7 (23)
135	FDA 71-33	11.0	33.1	36.5	39.3	49.2	53.9 (20)
136	FDA 71-33	50.0	30.7	35.0	36.1	45.4	50.9 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEAR LABORATORIES, INC.

Group 131 | Appendix II | Date September 29, 1972

Material Sham | Reproduction Data in Mice (Individual) | Laboratory No. 0901

Dose 0.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 1391	NP	3	0						----	
S 1392	P	16	12	12		7	5		0.54	
S 1393	NP	10	0						----	
S 1394	NP	9	0						----	
S 1395	P	13	11	11		4	7		0.58	
S 1396	P	20	15	12		7	5	3	0.76	
S 1397	P	6	1	1		1	0		1.15	
S 1398	NP	2	0						----	
S 1399									----	Not Assigned
S 1400	NP	9	0						----	
S 1401	P	18	15	15		3	12		0.88	
S 1402	P	15	13	13		4	9		0.90	
S 1403	P	19	16	12		5	7	4	0.88	
S 1404	P	7	2	2		2	0		1.04	
S 1405	P	20	13	13		8	5		0.88	
S 1406	P	19	13	13		6	7		0.97	
S 1407	P	20	13	13		7	6		0.94	
S 1408	P	19	14	13	1	10	3		0.84	
S 1409	P	14	13	11		7	4	2	1.22	
S 1410	P	17	12	12		5	7		1.04	
S 1411	P	14	11	11		7	4		0.89	
S 1412	P	17	11	11		6	5		1.17	
S 1413	P	14	12	12		8	4		0.83	
S 1414	P	16	13	10		8	2	3	1.01	
S 1415	P	17	13	13		7	6		0.83	

Continued on next page

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAR LABORATORIES, INC.

Group 131 | Appendix II | Date September 29, 1972

Material Sham | Reproduction Data in Mice (Individual) | Laboratory No. 0901

Dose 0.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Continued										
1416	NP	8	0						----	
1417	P	13	11	8		5	3	3	0.79	
1418	NP	8	0						----	
1419	P	15	14	11		2	9	3	0.89	
1420	NP	1	0						----	Died Day 14

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA. A LABORATORIES, INC.

Group 132

Material Aspirin

Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date Septemb 29, 1972

Laboratory No. 0901

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 1391	NP	7	0						----	
A 1392									----	Not Assigned
A 1393	P	19	16	16		14	2		0.74	
A 1394									----	Not Assigned
A 1395	NP	5	0						----	
A 1396	P	19	15	15		8	7		0.81	
A 1397	NP	6	0						----	
A 1398	P	15	13	13		6	7		0.80	
A 1399	P	18	14	14		7	7		0.77	
A 1400	P	12	10	10		5	5		0.92	
A 1401	P	20	17	16	1	3	13		0.67	
A 1402	P	14	13	10	1	6	4	2	0.85	
A 1403	P	16	15	15		7	8		1.00	
A 1404	P	18	12	12		9	3		0.87	
A 1405	P	16	12	11		5	6	1	0.86	
A 1406	P	13	10	9		6	3	1	1.12	
A 1407	P	19	12	11		3	8	1	0.82	
A 1408	P	22	14	14		4	10		0.90	
A 1409	P	15	15	12	1	6	6	2	0.76	
A 1410	P	18	15	12	1	6	6	2	0.87	
A 1411	P	21	13	12		5	7	1	0.72	
A 1412	P	20	16	15	1	6	9		0.85	
A 1413	P	14	12	10		2	8	2	0.70	
A 1414	P	7	4			--	--	4	----	
A 1415	P	16	11	11		7	4		0.89	
A 1416	NP	8	0						----	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEAR LABORATORIES, INC.

Group 131

Appendix II

Date September 29, 1972

Material FDA 71-33

Reproduction Data in Mice (Individual)

Laboratory No. 0901 t

Dose 0.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 1001	P	12	11	10		6	4	1	0.83	
T 1002	P	16	12	12		8	4		0.95	
T 1003	P	16	12	12		8	4		0.86	
T 1004	P	17	13	13		5	8		0.93	
T 1005	P	16	12	12		6	6		0.85	
T 1006	P	18	13	12		5	7	1	0.87	
T 1007	P	15	11	10	1	5	5		0.83	
T 1008	P	20	14	10	1	5	5	3	0.76	
T 1009	P	14	12	12		8	4		0.70	
T 1010	P	16	11	9		6	3	2	0.87	
T 1011	NP	0	0						----	
T 1012	NP	0	0						----	
T 1013	P	13	12	10		5	5	2	0.77	
T 1014	P	21	14	12		7	5	2	0.93	
T 1015	P	17	15	15		6	9		1.16	
T 1016	P	15	12	9		5	4	3	0.88	
T 1017	P	19	12	9		4	5	3	0.89	
T 1018	P	18	15	14	1	9	5		0.89	
T 1019	P	14	9	4		2	2	5	1.01	
T 1020	P	15	12	11		4	7	1	0.81	
T 1021	P	15	11	11		4	7		0.84	
T 1022	P	13	11	8	1	3	5	2	0.75	
T 1023	P	13	10	9		5	4	1	0.91	
T 1024	P	14	11	10		9	1	1	1.00	
T 1025	P	19	14	14		10	4		0.85	
T 1026	NP	9	0						----	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA LABORATORIES, INC.

Group 134 | Appendix II | Date September 29, 1972

Material FDA 71-33 | Reproduction Data in Mice (Individual) | Laboratory No. 0901 t

Dose 2.3 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 1031	P	16	12	11	1	5	6		0.89	
T 1032	NP	2	0						----	
T 1033	P	21	15	11	1	6	5	3	0.69	
T 1034	P	17	13	12		8	4	1	0.91	
T 1035	P	15	14	13		10	3	1	0.84	
T 1036	P	15	11	11		6	5		0.87	
T 1037	P	16	12	12		7	5		0.97	
T 1038	P	13	10	9		3	6	1	0.97	
T 1039	P	15	12	10	1	5	5	1	0.93	
T 1040	P	16	12	12		8	4		1.09	
T 1041	P	19	13	13		6	7		0.84	
T 1042	P	19	13	13		9	4		0.86	
T 1043	P	17	13	13		8	5		0.81	
T 1044	P	17	11	10		4	6	1	0.88	
T 1045	P	14	12	11		6	5	1	0.96	
T 1046	P	17	11	11		4	7		0.94	
T 1047	P	23	17	15	2	7	8		0.89	
T 1048	P	15	12	11		4	7	1	0.93	
T 1049	P	17	17	15	1	7	8	1	0.76	
T 1050	NP	9	0						----	
T 1051	P	15	11	11		5	6		0.99	
T 1052	P	19	12	10		5	5	2	0.94	
T 1053	P	21	17	17		11	6		0.83	
T 1054	P	15	7	5		3	2	2	0.90	
T 1055	P	17	13	13		6	7		0.96	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA. H LABORATORIES, INC.

Group 135 | Appendix II | Date September 29, 1972

Material FDA 71-33 | Reproduction Data in Mice (Individual) | Laboratory No. 0901 t

Dose 11.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 1061	P	13	5	5		2	3		0.92	
T 1062	P	12	11	9		5	4	2	0.77	
T 1063	P	22	14	14		7	7		0.78	
T 1064	P	17	13	13		7	6		0.95	
T 1065	P	16	12	12		6	6		0.90	
T 1066	P	13	12	11		2	9	1	1.03	
T 1067	P	19	15	12		4	8	3	0.79	
T 1068	NP	4	0						----	
T 1069	P	16	14	12		6	6	2	0.96	
T 1070	P	15	14	10		4	6	4	0.87	
T 1071	P	8	5	5		2	3		0.92	
T 1072	P	17	14	12		9	3	2	0.95	
T 1073	P	14	9	8		5	3	1	0.88	
T 1074	P	10	6	6		5	1		0.97	
T 1075	P	19	15	13		6	7	2	0.86	
T 1076	P	10	9	8		2	6	1	0.94	
T 1077	P	16	13	13		5	8		0.72	
T 1078	NP	3	0						----	
T 1079	P	15	11	11		8	3		0.92	
T 1080	P	17	18	16		9	7	2	0.94	
T 1081	P	21	16	16		6	10		0.88	
T 1082	P	19	19	17	1	4	13	1	0.78	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA[illegible] LABORATORIES, INC.

Group 136

Appendix II

Date September 29, 1972

Material FDA 71-33

Reproduction Data in Mice (Individual)

Laboratory No. 0901 t

Dose 50.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 1091	P	19	15	15		6	9		0.79	
T 1092	P	16	11	11		5	6		0.71	
T 1093	P	14	11	11		5	6		0.77	
T 1094	P	17	12	11	1	3	8		0.90	
T 1095	P	12	8	8		5	3		0.92	
T 1096	P	22	18	15	1	8	7	2	0.75	
T 1097	P	14	13	12	1	6	6		0.76	
T 1098	P	16	11	10		5	5	1	0.69	
T 1099	P	14	11	11		1	10		0.79	
T 1100	P	12	13	8		2	6	5	1.17	
T 1101	P	19	15	15		11	4		0.83	
T 1102	P	10	7	5		2	3	2	0.92	
T 1103	P	14	15	15		11	4		0.90	
T 1104	P	19	16	13	2	7	6	1	0.86	
T 1105	P	22	12	12		7	5		0.94	
T 1106	NP	7	0						----	
T 1107	NP	7	0						----	
T 1108	P	16	12	12		5	7		0.93	
T 1109	P	19	13	12		5	7	1	0.80	
T 1110	P	17	12	11	1	6	5		0.95	
T 1111	NP	3	0						----	
T 1112	NP	11	0						----	
T 1113	P	10	9	8	1	5	3		0.93	
T 1114	P	17	13	10	1	6	4	2	0.82	
T 1115	P	18	16	16		7	9		0.89	

* P = Pregnant; NP = Not Pregnant

031

Food and Drug Research Laboratories
INCORPORATED

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DRAFT REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date September 29, 1972

Laboratory No. 0902 t
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-33 (Stannous Chloride)

Examination Requested: Teratologic evaluation of FDA 71-33 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 50 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the fourteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge, Ph.D.
Vice President

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any members of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

FOOD AND DRUG RESEAR LABORATORIES, INC.

Groups: 131 through 136

Material: FDA 71-33

Date September 29, 1972

Laboratory No. 0902 t

Table 1

Fate Summary
(Rats)

Group	Material	Dose** mg/kg	Total Mated	Total Pregnant	At Term Surviving (Total)	At Term Number Pregnant
131	Sham	0.0	24	20	24	20
132	Aspirin*	250.0	24	24	24	24
133	FDA 71-33	0.5	24	23	23	22
134	FDA 71-33	2.3	24	22	24	22
135	FDA 71-33	11.0	24	23	24	23
136	FDA 71-33	50.0	24	24	24	24

* Positive Control at 250.0 mg/kg
** Administered as a water solution; 1.0 ml per kg of body weight

Group: 1 through 136

Material: FDA 71-33

Tab 2
Reproduction Data
(Rats)

Date: September 29, 972

Laboratory No.: 0902 t

Group:	131	132	133	134	135	136
Dose (mg/kg):	Sham	Aspirin**	0.5	2.3	11.0	50.0
Pregnancies						
Total No.	20	24	23	22	23	24
Died or Aborted (before Day 20)	0	0	1	0	0	0
To term (on Day 20)	20	24	22	22	23	24
Corpora Lutea						
Total No.	268	281	279	267	300	293
Average/dam mated	11.2	11.7	11.6	11.1	12.5	12.2
Live Litters						
Total No.*	20	19	22	22	23	24
Implant Sites						
Total No.	221	269	260	247	282	276
Average/dam*	11.1	11.2	11.8	11.2	12.3	11.5
Resorptions						
Total No.*	8	94	8	5	7	5
Dams with 1 or more sites resorbed	6	12	6	4	6	4
Dams with all sites resorbed	0	4	0	0	0	0
Per cent partial resorptions	30.0	50.0	27.3	18.2	26.1	16.7
Per cent complete resorptions	--	16.7	--	--	--	--
Live Fetuses						
Total No.	213	174	249	242	275	271
Average/dam*	10.7	7.25	11.3	11.0	12.0	11.3
Sex ratio (M/F)	0.84	0.78	0.92	0.95	0.98	1.13
Dead Fetuses						
Total No.*	0	1	3	--	--	--
Dams with 1 or more dead	--	1	2	--	--	--
Dams with all dead	--	0	0	--	--	--
Per cent partial dead	--	4.17	9.09	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.55	2.29	3.55	3.67	3.62	3.71

*Includes only those dams examined at term.
**Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEAR LABORATORIES, INC.

Groups: 131 through 136

Material: FDA 71-33

Date September 29, 1972

Laboratory No. 0902 t

Table 1

Fate Summary
(Rats)

Group	Material	Dose** mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
131	Sham	0.0	24	20	24	20
132	Aspirin*	250.0	24	24	24	24
133	FDA 71-33	0.5	24	23	23	22
134	FDA 71-33	2.3	24	22	24	22
135	FDA 71-33	11.0	24	23	24	23
136	FDA 71-33	50.0	24	24	24	24

* Positive Control at 250.0 mg/kg
** Administered as a water solution; 1.0 ml per kg of body weight

Group: 131 through 136

Material: FDA 71-33

Tab 12
Reproduction Data
(Rats)

Date: September 29, 1972

Laboratory No.: 0902 t

Group:	131	132	133	134	135	136
Dose (mg/kg):	Sham	Aspirin**	0.5	2.3	11.0	50.0
Pregnancies						
Total No.	20	24	23	22	23	24
Died or Aborted (before Day 20)	0	0	1	0	0	0
To term (on Day 20)	20	24	22	22	23	24
Corpora Lutea						
Total No.	268	281	279	267	300	293
Average/dam mated	11.2	11.7	11.6	11.1	12.5	12.2
Live Litters						
Total No.*	20	19	22	22	23	24
Implant Sites						
Total No.	221	269	260	247	282	276
Average/dam*	11.1	11.2	11.8	11.2	12.3	11.5
Resorptions						
Total No.*	8	94	8	5	7	5
Dams with 1 or more sites resorbed	6	12	6	4	6	4
Dams with all sites resorbed	0	4	0	0	0	0
Per cent partial resorptions	30.0	50.0	27.3	18.2	26.1	16.7
Per cent complete resorptions	--	16.7	--	--	--	--
Live Fetuses						
Total No.	213	174	249	242	275	271
Average/dam*	10.7	7.25	11.3	11.0	12.0	11.3
Sex ratio (M/F)	0.84	0.78	0.92	0.95	0.98	1.13
Dead Fetuses						
Total No.*	0	1	3	--	--	--
Dams with 1 or more dead	--	1	2	--	--	--
Dams with all dead	--	0	0	--	--	--
Per cent partial dead	--	4.17	9.09	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.55	2.29	3.55	3.67	3.62	3.71

*Includes only those dams examined at term.
**Positive Control: 250.0 mg/kg.

Group: 131 through 136

Material: FDA 71-33

Tab 2
Reproduction Data
(Rats)

Date: September 29, 972

Laboratory No.: 0902 t

Group:	131	132	133	134	135	136
Dose (mg/kg):	Sham	Aspirin**	0.5	2.3	11.0	50.0
Pregnancies						
Total No.	20	24	23	22	23	24
Died or Aborted (before Day 20)	0	0	1	0	0	0
To term (on Day 20)	20	24	22	22	23	24
Corpora Lutea						
Total No.	268	281	279	267	300	293
Average/dam mated	11.2	11.7	11.6	11.1	12.5	12.2
Live Litters						
Total No.*	20	19	22	22	23	24
Implant Sites						
Total No.	221	269	260	247	282	276
Average/dam*	11.1	11.2	11.8	11.2	12.3	11.5
Resorptions						
Total No.*	8	94	8	5	7	5
Dams with 1 or more sites resorbed	6	12	6	4	6	4
Dams with all sites resorbed	0	4	0	0	0	0
Per cent partial resorptions	30.0	50.0	27.3	18.2	26.1	16.7
Per cent complete resorptions	--	16.7	--	--	--	--
Live Fetuses						
Total No.	213	174	249	242	275	271
Average/dam*	10.7	7.25	11.3	11.0	12.0	11.3
Sex ratio (M/F)	0.84	0.78	0.92	0.95	0.98	1.13
Dead Fetuses						
Total No.*	0	1	3	--	--	--
Dams with 1 or more dead	--	1	2	--	--	--
Dams with all dead	--	0	0	--	--	--
Per cent partial dead	--	4.17	9.09	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.55	2.29	3.55	3.67	3.62	3.71

*Includes only those dams examined at term.
**Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 131 through 136 Date September 29, 1972

Material FDA 71-33 Laboratory No. 0902 t

Table 3-a
Summary of Soft Tissue Abnormalities
(Rats)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
132	Aspirin*	250.0	A 2396	1	Exencephaly; spina bifida; enterohepatocele
			A 2402	2	Exencephaly; spina bifida
			A 2403	2	Exencephaly
			A 2404	4	Exencephaly; spina bifida
			A 2406	2	Exencephaly; spina bifida
			A 2408	1	Exencephaly; spina bifida
				1	Exencephaly; enterohepatocele
				1	Exencephaly
				1	Exencephaly; enterohepatocele
			A 2413	1	Exencephaly; spina bifida
				1	Enterohepatocele

* Positive Control at 250.0 mg/kg

FOOD AND DRUG RESEA[illegible] LABORATORIES, INC.

Groups 131 through 136

Species Rats

Date September 29, 1972

Laboratory No. 0902 t

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	11	15	20**
		mg/kg	g				
131	Sham	0.0	241	259	276	297	365 (20)
132	Aspirin***	250.0	241	260	270	288	330 (24)
133	FDA 71-33	0.5	234	253	274	297	363 (22)
134	FDA 71-33	2.3	339	258	278	301	367 (22)
135	FDA 71-33	11.0	242	261	278	303	357 (23)
136	FDA 71-33	50.0	237	254	272	292	362 (24)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control:

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEAR LABORATORIES, INC.

Group 131

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 29, 1972

Laboratory No. 0902

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 2391	NP	0	0						----	
S 2392	P	10	10	10		5	5		3.45	
S 2393	P	12	12	12		6	6		3.38	
S 2394	P	12	12	12		4	8		3.29	
S 2395	P	16	16	16		8	8		4.13	
S 2396	P	12	12	12		3	9		3.40	
S 2397	P	10	11	10		5	5	1	3.28	
S 2398	NP	10	0						----	
S 2399	P	16	16	15		5	10	1	3.76	
S 2400	P	9	9	8		3	5	1	3.59	
S 2401	NP	11	0						----	
S 2402	P	9	9	9		4	5		3.76	
S 2403	NP	12	0						----	
S 2404	P	14	14	14		9	5		3.54	
S 2405	P	8	3	3		1	2		3.40	
S 2406	P	13	13	13		7	6		3.52	
S 2407	P	10	10	10		5	5		3.48	
S 2408	P	12	12	12		4	8		3.81	
S 2409	P	13	7	6		3	3	1	4.48	
S 2410	P	13	13	11		3	8	2	3.61	
S 2411	P	9	9	9		5	4		3.70	
S 2412	P	11	7	7		5	2		3.56	
S 2413	P	11	11	9		4	5	2	3.64	
S 2414	P	15	15	15		8	7		3.29	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 132 | Appendix II | Date September 29, 1972

Material Aspirin | Reproduction Data in Rats (Individual) | Laboratory No. 0902

Dose 250.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 2391	P	12	12	12		1	11		3.00	
A 2392	P	12	12	12		7	5		2.14	
A 2393	P	15	13	13		8	5		2.07	
A 2394	P	11	11	11		7	4		4.07	
A 2395	P	11	11					11	----	
A 2396	P	11	11	1		0	1	10	1.00	
A 2397	P	12	12	12		6	6		2.73	
A 2398	P	13	13					13	----	
A 2399	P	13	13	12		3	9	1	2.13	
A 2400	P	12	12	1		1	0	11	1.50	
A 2401	P	11	11		1			10	----	
A 2402	P	13	13	9		4	5	4	2.39	
A 2403	P	11	11	11		7	4		1.93	
A 2404	P	11	11	11		4	7		3.20	
A 2405	P	13	8					8	----	
A 2406	P	10	8	2		1	1	6	1.80	
A 2407	P	12	12					12	----	
A 2408	P	10	10	8		2	6	2	2.06	
A 2409	P	14	14	14		5	9		2.90	
A 2410	P	11	11	11		8	3		1.93	
A 2411	P	8	8	8		3	5		2.65	
A 2412	P	9	8	2		1	1	6	1.90	
A 2413	P	13	11	11		5	6		1.84	
A 2414	P	13	13	13		3	10		2.24	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEAR. (LABORATORIES, INC.

Group 133 | Appendix II | Date September 29, 1972

Material FDA 71-33 | Reproduction Data in Rats (Individual) | Laboratory No. 0902 t

Dose 0.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 2001	P	10	10	7		3	4	3	3.40	
T 2002	P	12	12	12		7	5		3.98	
T 2003	P	13	13	12		6	6	1	3.31	
T 2004	P	11	11	11		9	2		3.49	
T 2005	P	10	10	10		--	--		----	Died Day 19
T 2006	P	10	10	9	1	5	4		3.91	
T 2007	P	11	11	11		6	5		3.89	
T 2008	P	14	14	14		4	10		3.29	
T 2009	P	11	11	11		3	8		3.86	
T 2010	P	10	10	10		6	4		3.77	
T 2011	P	10	10	10		6	4		3.41	
T 2012	P	10	10	10		4	6		3.75	
T 2013	P	13	13	12		5	7	1	3.94	
T 2014	P	12	12	12		7	5		3.43	
T 2015	P	13	13	12		3	9	1	3.41	
T 2016	P	11	11	11		6	5		3.77	
T 2017	P	13	13	13		4	9		3.30	
T 2018	P	14	14	14		6	8		3.13	
T 2019	P	17	17	14	2	7	7	1	3.21	
T 2020	P	11	11	11		3	8		3.53	
T 2021	P	12	12	12		6	6		3.62	
T 2022	NP	9	0						----	
T 2023	P	7	7	7		3	4		3.20	
T 2024	P	15	15	14		10	4	1	3.51	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAR * LABORATORIES, INC.

Group 133

Appendix II

Date September 29, 1972

Material FDA 71-33

Reproduction Data in Rats (Individual)

Laboratory No. 0902 t

Dose 0.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 2001	P	10	10	7		3	4	3	3.40	
T 2002	P	12	12	12		7	5		3.98	
T 2003	P	13	13	12		6	6	1	3.31	
T 2004	P	11	11	11		9	2		3.49	
T 2005	P	10	10	10		--	--		----	Died Day 19
T 2006	P	10	10	9	1	5	4		3.91	
T 2007	P	11	11	11		6	5		3.89	
T 2008	P	14	14	14		4	10		3.29	
T 2009	P	11	11	11		3	8		3.86	
T 2010	P	10	10	10		6	4		3.77	
T 2011	P	10	10	10		6	4		3.41	
T 2012	P	10	10	10		4	6		3.75	
T 2013	P	13	13	12		5	7	1	3.94	
T 2014	P	12	12	12		7	5		3.43	
T 2015	P	13	13	12		3	9	1	3.41	
T 2016	P	11	11	11		6	5		3.77	
T 2017	P	13	13	13		4	9		3.30	
T 2018	P	14	14	14		6	8		3.13	
T 2019	P	17	17	14	2	7	7	1	3.21	
T 2020	P	11	11	11		3	8		3.53	
T 2021	P	12	12	12		6	6		3.62	
T 2022	NP	9	0						----	
T 2023	P	7	7	7		3	4		3.20	
T 2024	P	15	15	14		10	4	1	3.51	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 135 | Appendix II | Date September 29, 1972

Material FDA 71-33 | Reproduction Data in Rats (Individual) | Laboratory No. 0902 t

Dose 11.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 2061	P	11	11	10		7	3	1	4.42	
T 2062	P	12	12	12		5	7		3.58	
T 2063	P	14	14	14		6	8		3.68	
T 2064	P	14	14	13		6	7	1	3.69	
T 2065	P	15	14	14		6	8		3.84	
T 2066	P	13	12	12		4	8		3.92	
T 2067	P	11	11	11		6	5		3.65	
T 2068	P	14	14	14		6	8		3.46	
T 2069	P	14	14	14		9	5		3.39	
T 2070	P	11	11	11		6	5		3.85	
T 2071	P	12	11	10		4	6	1	3.82	
T 2072	P	9	9	9		1	8		3.46	
T 2073	P	12	12	12		5	7		3.32	
T 2074	P	15	12	12		9	3		3.66	
T 2075	P	12	12	12		3	9		3.46	
T 2076	P	14	12	11		7	4	1	3.36	
T 2077	P	11	11	9		3	6	2	3.61	
T 2078	P	12	12	12		6	6		4.04	
T 2079	P	11	11	11		6	5		3.18	
T 2080	P	13	13	12		6	6	1	3.56	
T 2081	P	12	12	12		9	3		2.40	
T 2082	P	16	16	16		9	7		3.80	
T 2083	NP	10	0						----	
T 2084	P	12	12	12		7	5		4.06	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA H LABORATORIES, INC.

Group 136 | Appendix II | Date September 29, 1972

Material FDA 71-33 | Reproduction Data in Rats (Individual) | Laboratory No. 0902 t

Dose 50.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 2091	P	14	14	14		6	8		3.74	
T 2092	P	15	15	14		7	7	1	3.64	
T 2093	P	11	8	7		4	3	1	3.64	
T 2094	P	10	10	9		3	6	1	3.99	
T 2095	P	11	11	11		5	6		3.47	
T 2096	P	13	13	13		9	4		3.86	
T 2097	P	12	10	10		6	4		3.03	
T 2098	P	13	13	13		6	7		3.68	
T 2099	P	7	1	1		0	1		4.30	
T 2100	P	12	12	12		6	6		3.89	
T 2101	P	10	10	10		6	4		4.00	
T 2102	P	10	10	10		6	4		3.78	
T 2103	P	14	14	14		8	6		3.56	
T 2104	P	13	13	13		6	7		3.36	
T 2105	P	11	11	11		5	6		4.09	
T 2106	P	15	15	15		9	6		3.43	
T 2107	P	13	12	12		7	5		3.50	
T 2108	P	11	11	11		3	8		3.81	
T 2109	P	12	12	12		9	3		3.71	
T 2110	P	13	13	13		8	5		3.72	
T 2111	P	14	14	14		5	9		3.78	
T 2112	P	15	13	11		8	3	2	4.22	
T 2113	P	14	11	11		5	6		3.16	
T 2114	P	10	10	10		7	3		3.65	

* P = Pregnant; NP = Not Pregnant

Food and Drug Research Laboratories
INCORPORATED

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REPORT
DRAFT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date September 29, 1972

Laboratory No. 0903 t
Contract No. FDA 71-260

Sample: White Crystalline Material

Marking: FDA 71-33 (Stannous Chloride)

Examination Requested: Teratologic evaluation of FDA 71-33 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 50 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the fourteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEAR LABORATORIES, INC.

Groups: 131 through 136

Material: FDA 71-33

Date September 29, 1972

Laboratory No. 0903 t

Table 1

Fate Summary
(Hamsters)

Group	Material	Dose ** mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
131	Sham	0.0	22	21	22	21
132	Aspirin*	250.0	22	22	22	22
133	FDA 71-33	0.5	22	21	22	21
134	FDA 71-33	2.3	22	20	22	20
135	FDA 71-33	11.0	22	21	22	21
136	FDA 71-33	50.0	22	21	22	21

* Positive Control at 250.0 mg/kg
** Administered as a water solution; 1.0 ml per kg of body weight

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Group: 1 through 136

Material: FDA 71-33

Tab 2
Reproduction Data
(Hamsters)

Date: September 29, 72

Laboratory No.: 0903 t

Group:	131	132	133	134	135	136
Dose (mg/kg):	Sham	Aspirin**	0.5	2.3	11.0	50.0
Pregnancies						
Total No.	21	22	21	20	21	21
Died or Aborted (before Day 14)	0	0	0	0	0	0
To term (on Day 14)	21	22	21	20	21	21
Corpora Lutea						
Total No.	334	374	339	336	360	348
Average/dam mated	15.2	17.0	16.1	16.0	16.4	15.8
Live Litters						
Total No.*	20	22	21	19	21	21
Implant Sites						
Total No.	291	308	281	280	297	279
Average/dam*	13.9	14.0	13.4	14.0	14.1	13.3
Resorptions						
Total No.*	21	31	32	19	8	15
Dams with 1 or more sites resorbed	9	14	14	11	6	7
Dams with all sites resorbed	1	--	--	--	--	--
Per cent partial resorptions	42.9	63.6	66.7	55.0	28.6	33.3
Per cent complete resorptions	4.76	--	--	--	--	--
Live Fetuses						
Total No.	269	276	249	248	286	264
Average/dam*	12.8	12.6	11.9	12.4	13.6	12.6
Sex ratio (M/F)	0.52	0.54	0.53	0.78	0.79	0.59
Dead Fetuses						
Total No.*	1	1	--	13	3	--
Dams with 1 or more dead	1	1	--	2	2	--
Dams with all dead	--	--	--	1	--	--
Per cent partial dead	4.76	4.55	--	10.0	9.52	--
Per cent all dead	--	--	--	5.00	--	--
Average Fetus Weight, g	1.66	1.69	1.67	1.69	1.67	1.69

*Includes only those dams examined at term.

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 131 through 136

Laboratory No. 0903 t

Table 3

Material FDA 71-33

Date September 29, 1972

Summary of Skeletal Findings*
(Hamsters)

Findings	Group No.	131	132	133	134	135	136
	Dose (mg/kg)	Sham	Aspirin**	0.5	2.3	11.0	50.0
Live Fetuses Examined (at term)		188/20	192/22	174/21	172/19	198/21	186/21
Sternebrae							
Incomplete oss.		129/20	144/22	120/21	101/17	104/20	113/21
Scrambled							
Bipartite		28/16	31/16	22/14	21/11	28/15	20/11
Fused		2/1			1/1		
Extra							1/1
Missing		43/13	34/17	28/12	18/9	37/12	71/15
Other							
Ribs							
Incomplete oss.							
Fused/split							
Wavy							
Less than 12							
More than 13		15/12	50/22	43/17	40/18	38/17	25/17
Other							
Vertebrae							
Incomplete oss.		2/2					1/1
Scrambled							
Fused							
Extra ctrs. oss.							
Scoliosis			1/1				
Tail defects							
Other							
Skull							
Incomplete closure							
Missing							
Craniostosis							
Other							
Extremities							
Incomplete oss.				2/2			1/1
Missing							
Extra							
Miscellaneous							
Hyoid; missing		1/1	1/1		2/2	1/1	5/3
Hyoid; reduced			1/1	1/1	1/1		

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte
** Positive control at 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 131 through 136

Date September 29, 1972

Material FDA 71-33

Laboratory No. 0903 t

Table 3-a

Summary of Soft Tissue Abnormalities
(Hamsters)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
133	FDA 71-33	0.5	T 3018	1	Meningoencephalocele

FOOD AND DRUG RESEAR' LABORATORIES, INC.

Groups 131 through 136

Species Hamsters

Date September 29, 1972

Laboratory No. 0903 t

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	8	10	14**
		mg/kg	g				
131	Sham	0.0	134.2	138.8	141.8	153.7	174.7 (21)
132	Aspirin***	250.0	134.2	139.5	142.9	155.0	174.9 (22)
133	FDA 71-33	0.5	127.0	133.4	136.8	146.8	169.5 (21)
134	FDA 71-33	2.3	133.6	142.7	143.8	153.1	175.7 (20)
135	FDA 71-33	11.0	135.8	141.3	144.0	155.5	177.2 (21)
136	FDA 71-33	50.0	134.8	138.3	141.9	152.6	171.6 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

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Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation; the controls were sham-treated.

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of skeletal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 131

Appendix II

Date September 29, 197

Material Sham

Reproduction Data in Hamsters (Individual)

Laboratory No. 0903

Dose 0.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 3391	P	13	10	10		3	7		1.87	
S 3392	P	16	12	12		7	5		1.86	
S 3393	P	16	13	13		6	7		1.85	
S 3394	P	--**	16	16		5	11		1.70	
S 3395	P	18	9	8		2	6	1	1.55	
S 3396	P	16	12	11		4	7	1	1.68	
S 3397	P	15	11					11	----	
S 3398	NP	11	0						----	
S 3399	P	23	20	18		10	8	2	1.60	
S 3400	P	9	8	6		4	2	2	1.72	
S 3401	P	17	17	17		8	9		1.36	
S 3402	P	12	11	11		2	9		1.28	
S 3403	P	16	16	15		4	11	1	1.59	
S 3404	P	17	15	14		1	13	1	1.32	
S 3405	P	21	21	21		9	12		1.60	
S 3406	P	21	19	19		5	14		1.58	
S 3407	P	19	17	16	1	--	--		1.70	
S 3408	P	15	14	13		1	12	1	1.69	
S 3409	P	14	12	11		3	8	1	1.89	
S 3410	P	12	11	11		4	7		1.72	
S 3411	P	15	12	12		4	8		1.76	
S 3412	P	18	15	15		5	10		1.93	

* P = Pregnant; NP = Not Pregnant

** Incomplete ovary

FOOD AND DRUG RESEA. H LABORATORIES, INC.

Group 132

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date Septemb. 29, 1972

Laboratory No. 0903

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 3391	P	15	13	13		5	8		1.78	
A 3392	P	15	13	13		6	7		1.76	
A 3393	P	17	13	13		3	10		1.74	
A 3394	P	21	14	13		6	7	1	1.69	
A 3395	P	18	15	15		4	11		1.44	
A 3396	P	16	12	12		7	5		1.67	
A 3397	P	22	18	17	1	3	14		1.54	
A 3398	P	17	13	10		1	9	3	1.42	
A 3399	P	15	11	9		1	8	2	1.86	
A 3400										Not Assigned
A 3401	P	22	18	17		11	6	1	1.71	
A 3402	P	15	12	8		2	6	4	1.79	
A 3403	P	18	18	16		2	14	2	1.59	
A 3404	P	17	14	12		4	8	2	1.73	
A 3405	P	15	14	13		2	11	1	1.54	
A 3406	P	21	18	17		5	12	1	1.54	
A 3407	P	22	17	17		6	11		1.75	
A 3408	P	14	13	10		2	8	3	1.38	
A 3409	P	16	10	7		2	5	3	1.77	
A 3410	P	16	16	12		5	7	4	1.82	
A 3411	P	15	12	11		6	5	1	1.97	
A 3412	P	13	12	9		6	3	3	1.83	
A 3413	P	14	12	12		8	4		1.96	

* P = Pregnant; NP = Not Pregnant

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FOOD AND DRUG RESEA H LABORATORIES, INC.

Group 133

Appendix II

Date September 29, 1972

Material FDA 71-33

Reproduction Data in Hamsters (Individual)

Laboratory No. 0903 t

Dose 0.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 3001	P	16	11	10		3	7	1	1.44	
T 3002	P	23	16	13		5	8	3	1.60	
T 3003	P	15	11	10		4	6	1	1.77	
T 3004	P	17	12	11		3	8	1	1.85	
T 3005	P	13	9	9		2	7		1.76	
T 3006	NP	12	0						----	
T 3007	P	19	14	14		5	9		1.82	
T 3008	P	22	18	18		7	11		1.45	
T 3009	P	16	12	11		2	9	1	1.61	
T 3010	P	--**	14	13		3	10	1	1.56	
T 3011	P	15	16	1		0	1	15	1.23	
T 3012	P	15	11	9		5	4	2	1.75	
T 3013	P	13	12	11		6	5	1	1.80	
T 3014	P	18	18	16		6	10	2	1.66	
T 3015	P	17	17	16		5	11	1	1.68	
T 3016	P	17	16	16		2	14		1.55	
T 3017	P	17	15	14		7	7	1	1.75	
T 3018	P	15	14	13		4	9	1	1.63	
T 3019	P	14	10	10		4	6		1.85	
T 3020	P	13	10	9		3	6	1	1.89	
T 3021	P	16	11	11		4	7		1.76	
T 3022	P	16	14	14		6	8		1.68	

* P = Pregnant; NP = Not Pregnant
** Incomplete ovary

FOOD AND DRUG RESEA 'H LABORATORIES, INC.

Group 134

Appendix II

Date September 29, 1972

Material FDA 71-33

Reproduction Data in Hamsters (Individual)

Laboratory No. 0903 t

Dose 2.3 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 3031	P	16	12	10		5	5	2	1.76	
T 3032	P	15	11	11		9	2		1.76	
T 3033	P	13	10	9		4	5	1	1.66	
T 3034	NP	7	0						----	
T 3035	P	14	15	14		7	7	1	1.72	
T 3036	P	23	17	16		4	12	1	1.73	
T 3037	P	20	14	14		6	8		1.66	
T 3038	NP	14	0						----	
T 3039	P	13	13	10		7	3	3	1.92	
T 3040	P	16	16	16		8	8		1.72	
T 3041	P	17	16	15	1	7	8		1.66	
T 3042	P	23	19	19		6	13		1.35	
T 3043	P	17	15	12		2	10	3	1.75	
T 3044	P	21	12		12				----	
T 3045	P	19	17	14		4	10	3	1.50	
T 3046	P	17	15	15		5	10		1.59	
T 3047	P	--**	17	17		9	8		1.75	
T 3048	P	15	14	14		5	9		1.65	
T 3049	P	14	12	10		5	5	2	1.65	
T 3050	P	13	11	10		7	3	1	1.74	
T 3051	P	14	11	10		5	5	1	1.87	
T 3052	P	15	13	12		4	8	1	1.73	

* P = Pregnant; NP = Not Pregnant

** Incomplete ovary

FOOD AND DRUG RESEA. " LABORATORIES, INC.

Group 135

Material FDA 71-33

Dose 11.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date September 29, 1972

Laboratory No. 0903 t

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 3061	P	15	12	12		1	11		1.64	
T 3062	P	17	11	11		6	5		1.64	
T 3063	P	17	15	15		9	6		1.78	
T 3064	P	18	14	14		4	10		1.76	
T 3065	P	14	11	11		6	5		2.03	
T 3066	P	24	21	21		13	8		1.72	
T 3067	P	14	10	10		6	4		1.83	
T 3068	P	18	13	13		4	9		1.36	
T 3069	P	19	16	15		4	11	1	1.49	
T 3070	P	15	17	17		8	9		1.54	
T 3071	P	15	15	15		6	9		1.71	
T 3072	P	14	10	9		6	3	1	1.78	
T 3073	P	17	16	15	1	7	8		1.42	
T 3074	P	17	16	16		7	9		1.57	
T 3075	P	18	16	16		6	10		1.61	
T 3076	P	17	17	16		4	12	1	1.70	
T 3077	P	19	18	18		12	6		1.51	
T 3078	P	17	13	13		3	10		1.61	
T 3079	P	15	12	8	2	5	3	2	1.80	
T 3080	NP	10	0						----	
T 3081	P	15	11	10		5	5	1	1.84	
T 3082	P	15	13	11		4	7	2	1.78	

* P = Pregnant; NP = Not Pregnant

031

FOOD AND DRUG RESEA. LABORATORIES, INC.

Group 136

Material FDA 71-33

Dose 50.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date September 29, 1972

Laboratory No. 0903 t

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
T 3091	P	15	14	14		4	10		1.72	
T 3092	P	14	12	11		2	9	1	1.67	
T 3093	P	16	12	8		2	6	4	1.53	
T 3094	P	15	12	12		10	2		1.88	
T 3095	P	15	10	10		4	6		1.83	
T 3096	P	14	13	13		3	10		1.54	
T 3097	NP	11	0						----	
T 3098	P	20	17	17		6	11		1.72	
T 3099	P	17	14	13		4	9	1	1.62	
T 3100	P	18	17	17		7	10		1.66	
T 3101	P	18	7	6		2	4	1	1.81	
T 3102	P	16	15	15		2	13		1.91	
T 3103	P	16	15	14		5	9	1	1.63	
T 3104	P	18	17	17		4	13		1.67	
T 3105	P	16	15	13		6	7	2	1.59	
T 3106	P	17	16	16		3	13		1.65	
T 3107	P	19	16	16		7	9		1.58	
T 3108	P	15	16	11		6	5	5	1.17	
T 3109	P	14	9	9		5	4		1.99	
T 3110	P	14	11	11		7	4		1.83	
T 3111	P	14	10	10		6	4		1.79	
T 3112	P	16	11	11		3	8		1.78	

* P = Pregnant; NP = Not Pregnant

Food Protection Committee

Survey of Substances Generally Recognized as Safe

National Academy of Sciences, National Research Council, Washington, D. C.

(Pertinent section included in monograph text.)

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 488

FAO NUTRITION MEETINGS REPORT SERIES
No. 50

EVALUATION OF FOOD ADDITIVES

SOME ENZYMES, MODIFIED STARCHES, AND CERTAIN OTHER SUBSTANCES: TOXICOLOGICAL EVALUATIONS AND SPECIFICATIONS AND A REVIEW OF THE TECHNOLOGICAL EFFICACY OF SOME ANTIOXIDANTS

Fifteenth Report
of the Joint FAO/WHO Expert Committee
on Food Additives

Rome, 16–24 June 1971

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WORLD HEALTH ORGANIZATION
GENEVA
1972

amount of propylene glycol that could be liberated from the alginate ether. With this restriction an unconditional acceptable daily intake was established.

5.3.8 *Stearoyl lactylic acid, calcium and sodium salts*

These substances have been reviewed previously and tentative specifications were prepared at the thirteenth meeting of the Committee.[1] These have now been finalized and will be published. Previously there had been concern over the significance of some toxicological observations. However, stearoyl lactylates act in the same way as equivalent amounts of stearic acid and lactic acid when these are administered concomitantly. The production of "lipogranulomata" in animals fed high levels of stearoyl lactylates can be prevented by incorporation of additional unsaturated fatty acids into diets. This effect therefore appears to be the result of dietary imbalance and there may be a need to take into account the intake of stearic acid from all sources. The acceptable daily intake established was higher than that assigned in the previous evaluation.

5.3.9 *Tin and stannous chloride*

A specification for stannous chloride was prepared at the fourteenth meeting of the Committee.[2] Information is lacking on the various combinations in which tin is present in canned foods and beverages. The uptake of tin from the container by canned foods is a complex reaction, which may be influenced by the presence of ions such as nitrate, oxalate or potassium in the product. Further research should be directed toward determining the form in which tin is present in beverages, such as diluted citrus juices, and in canned foods, such as fruit and vegetables, where it is apparently bound to polyphenolic compounds or to proteins. Priority should be given to consideration of tin in beverages since it is these that have been reported to cause acute gastrointestinal troubles. The study of tin in canned foods is relevant to possible long-term toxic effects. The effect of tin on the organoleptic, keeping, and other qualities of foods is not fully understood and should be examined more thoroughly.

After extensive discussion of the significance of new laboratory data and further human epidemiological observations, the previous evaluation was confirmed, i.e., no ADI could be assigned, but there was no need to depart from the limits set by good manufacturing practice. However, further assessment will be needed when the results of work in progress become available.

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[1] See Annex 1, ref. 19.

[2] See Annex 1, ref. 24.

[CANCER RESEARCH 27, 1012-1018, July 1967]

Life Term Studies on the Effects of Arsenic, Germanium, Tin, and Vanadium on Spontaneous Tumors in Mice[1]

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SUMMARY

Mice fed a diet low in toxic trace elements and raised in an environment where contamination from extraneous trace elements was controlled were given small amounts (5 μg/ml.) of arsenite, germanate, stannous, or vanadyl ions in drinking water for their life times. Animals receiving arsenite had a significantly decreased incidence of all tumors, and of tumors of the lung, compared to those receiving tin, vanadium, and to controls. Fewer spontaneous tumors were also found in animals given germanium. Significant differences in the incidences of malignant tumors did not appear. None of the elements was carcinogenic in the doses given.

INTRODUCTION

During studies on the effects of small doses of various trace elements on the growth and survival of mice (1), we have observed that the incidences of visible spontaneous tumors were apparently affected by the ingestion of cadmium, lead, and nickel (8). Male mice receiving cadmium or lead salts (5 μg/ml.) in drinking water for their lifetimes exhibited significantly fewer tumors, especially in lung; female mice given nickel (5 μg/ml.) in water also had fewer tumors than did control animals and those receiving chromium (III) or titanium (IV) salts, and all were in lung. The present report considers the incidences of spontaneous tumors in mice of both sexes given trivalent arsenic, divalent tin, tetravalent germanium, and tetravalent vanadium salts for their lifetimes.

MATERIALS AND METHODS

The experimental conditions of a relatively metal-free environment has been reported in detail (1). Six hundred one cesarean-born white Swiss mice of the Charles River strain (CD-1), born in our laboratory from pregnant females were exposed from the time of weaning (20-22 days of age) until death to doubly deionized drinking water containing the essential trace metals manganese (10 μg/ml.), chromium (5 μg/ml.), cobalt (1 μg/ml.), copper (5 μg/ml.), zinc (50 μg/ml.), and molybdenum (1 μg/ml.) as the acetates, nitrate, or molybdate. Groups of 23-71 of these mice randomly selected from litters were given the additional elements at 5 μg/ml. in addition of water: sodium arsenite, sodium germanate, stannous chloride (stabilized with ascorbic acid), or vanadyl sulfate. The diet was composed of seal rye flour (60%), dry skim milk (30%), corn oil (9%), and sodium chloride (1%), to which 100 mg./kg. ferrous sulfate and a vitamin mixture (5%) were added. The diet contained 0.40 μg. arsenic/gm. (3), 0.32 μg. germanium/gm. (3), 0.28 μg. tin/gm. (7) and 3.2 μg. vanadium/gm. (3). Extensive precautions were taken to avoid extraneous metal contamination (9).

Dead animals were weighed and 542 were dissected. Gross anatomical lesions were recorded, and tissues of 431 outside abnormal were fixed in Bouin's solution, sectioned, and stain with hematoxylin and eosin. Microscopic examinations were made on heart, lung, kidney, liver, spleen, and on other organs considered grossly abnormal, as well as on visible tumors. A detailed search for microscopic tumors was conducted by serial sections; therefore, the data here presented represent only tumors visibly under a magnifying lens. Sixty-three animals dying during a weekend with gross autolytic changes or cannibalism were not included except for determination of life span. Statistical data were tested by chi-square analysis and by Student's t-test.

The following criteria were used in examining the series of "precancerous" lesions, found in lung or liver, were growths of atypical parenchymal cells lacking proper stroma, replacing normal epithelial cells in bronchus, alveolus, or hepatic cinus. Benign tumors were autonomous growths of well-differentiated cells which might be slightly atypical, but which did not metastasize or invade. Malignant tumors were autonomous destructive growths of highly atypical cells which sometimes metastasized to other organs or in the original organ.

RESULTS

The data from examination of the microscopic sections of analyzed according to the presence of clusters of cells in lung and liver appearing "precancerous," of circumscribed lymphosarcoma, or carcinoma, sarcomata, hemangiomata, lymphomata, leukemia and of other tumors benign and malignant in lung, liver, mammary glands, and other locations. As no significant differences appeared between incidences of tumors in the two sexes, they were grouped together.

In Table 1 are shown the numbers of benign and malignant tumors found in the various groups related to the number

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[2] Requests for reprints should be sent to Dr. H. A. Schroeder, 9 Belmont Avenue, Brattleboro, Vermont 05301.

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TABLE 1

Number of Deaths and Number of Tumors Found in Mice (Both Sexes) in Each Three-Month Period

Period (Months)	Control Deaths (No.)	Control Tumors No.	Control Tumors %	Arsenic Deaths (No.)	Arsenic Tumors (No.)	Arsenic Tumors %	Germanium Deaths (No.)	Germanium Tumors No.	Germanium Tumors %	Tin Deaths (No.)	Tin Tumors (No.)	Tin Tumors %	Vanadium Deaths (No.)	Vanadium Tumors No.	Vanadium Tumors %
9	10	1	10	6			9			5			5		
12	12	1 (11)*	8	2			11	3 (27)	30	9	1	11	2		
15	18	3	17	24			15	7 (11)	47	1	1	25	4	3 (34)	100
18	34	10 (51)	32	28	4 (27)	14	33	7 (11)	21	18	4 (23)	22	7	1 (11)	11
21	43	21 (31)	49	21	1	1	38	5 (23)	15	24	6 (11)	25	17	4 (23)	24
24	30	8	29	15	3 (11)	20	21	3 (11)	14	16	4 (11)	27	10	6 (21)	60
27	14	9 (61)	61	2	2 (22)	100	6			6	3 (11)	50	2	1 (11)	50
30	2	1	50	2	1 (11)	50	1			2	1 (11)	50	1		
33	1	1	100				2			2	2	100			
Total	170	55 (151)		103	11 (33)		131	25 (74)		86	22 (61)		47	15 (99)	
% of totals			32.4			10.7			19.1			25.6			32.0
Subjects not done	28			5			1			22			5		

Numbers in parentheses represent totals of malignant tumors.
Differs from control number $P < 0.0005$.
Differs from control number $P < 0.025$.

ards in each three-month period. Mice receiving arsenic or germanium showed significantly lower incidence of tumors than did controls, although a decreased incidence of malignant tumors was not statistically significant. Mice receiving tin and vanadium did not exhibit significantly decreased or increased incidence of tumors based on the total number of animals autopsied. Somewhat less tumors were found in those given germanium.

In Table 2 are shown deaths and tumors in mice dying between 9 and 24 months of age, the highest death rates being in this period. Although the percentages of deaths were quite similar in the groups, as were the percentages of tumors, there were significantly fewer tumors in the arsenic and germanium groups than in the controls, based on total number of animals autopsied. Organ distribution of tumors and their types are given in Table 3. There were significantly fewer adenomata of the lung in arsenic-fed mice and also fewer adenomata and carcinomata of the lung combined ($P < 0.00001$). Differences in other organs and types of tumors were not significant.

Another comparison was made on the incidence of tumors and "tumorous" lesions in the sections examined, excluding those in which sections were not taken (Table 4). Mice receiving arsenic and germanium had significantly fewer lesions than did controls. They also had fewer tumors. Significant differences were not found for malignant tumors.

The life spans of all mice receiving metals, which will be treated elsewhere (5), can be calculated from Table 1 for those deceased. Large numbers of early deaths of animals in any period do not occur. Significant differences in survival of all mice in the three-month period were not observed for the tin and vanadium groups. There were fewer males given arsenic alive at 18 months of age ($P < 0.025$) and fewer males given germanium ($P < 0.005$) compared to controls. At 21 months of age, there were fewer females given arsenic ($P < 0.05$). Other differences were not significant.

TABLE 2

Death Rates and Incidence of Spontaneous Tumors in Anticipated Mice Dying Between Three Elements during 15–24 Months of Age

Metal	No. of deaths	% all deaths	No. of tumors	% all tumors	% deaths with tumors
Control	113	66.5	39	70.9	34.5
Arsenic	67	65.1	34	72.3	51.0
Germanium	88	67.2	15	60.0	17.1
Tin	58	67.4	11	50.0	21.2
Vanadium	34	72.3	11	73.3	32.4

* Differs from control number $P < 0.001$.
Differs from control number $P < 0.01$.

Note: Between 12 and 24 months of age there were 76 deaths with 5 tumors in the arsenic group and 92 deaths with 31 tumors in the control group ($P < 0.0001$).

There were 140 of 198 control mice alive after 15 months of age (70.6%), of which 25 had tumors at death or at the arsenic-fed mice, 74 of 108 survived 15 months (68.5%), of which number 3 subsequently had pulmonary tumors ($P > 0.05$). There was no evidence of an earlier than usual appearance of tumors in one of the other three groups.

Kidney weights of the mice given arsenic and germanium are presented in Table 5. At one year and eighteen months, males fed arsenic or germanium were smaller than their controls, as were females fed germanium at eighteen months. The greatest decline in weight amounted to approximately 17.0–19.0%, or that expected. Similar changes were not found in the tin and vanadium fed animals, which weighed approximately the same as their controls at all ages.

TABLE 3

Types of Tumors in Various Organs of Mice Receiving Arsenic, Germanium, Tin, or Vanadium

Lesion	Control		Arsenic		Germanium		Tin		Vanadium	
	No.	%	No.	%	No.	%	No.	%	No.	%
Adenoma, benign										
Lung	20		2*		10		8		4	
Liver	7		0		2		1		1	
Mammary gland	2		4		1		0		0	
Subtotal	29	52.7	6	27.3	13	52.0	9	40.9	5	33.3
Malignant tumors										
Lung	6		4		1		3		4	
Liver	0		1		2		0		0	
Mammary gland	5		0		0		2		1	
Leukemia	2		3		1		0		3	
Lymphoma	2		0		0		0		0	
Hypernephroma	0		0		1		1		0	
Other	0		1*		0		1		1	
Subtotal	15	27.3	8	54.5	8	32.0	4	27.2	9	60.0
Miscellaneous										
Lymphoma, benign	1		0		0		1		0	
Other, benign	8		2		4		4		1	
Subtotal	11	20.0	2	18.2	4	16.0	5	31.8	1	6.7
Total	55	100	11	100	25	100	22	100	15	100
Precancerous lesions										
Lung	5		3		3		1		0	
Liver	1		1		4		2		1	

* Differs from control $P < 0.005$.

Note: Differences of percentages between other groups are not significant.

TABLE 4

Weight of Mice Given Germanium and Arsenic

Weeks of age	Control (mean ± SE)	Germanium (mean ± SE)	Arsenic (mean ± SE)	
Number of mice	34	31	34	
60	28.8 ± 0.33	37.1 ± 1.05	38.1 ± 0.7	71
90	35.2 ± 0.75	42.1 ± 1.36	43.8 ± 0.7	71
120	36.7 ± 0.36	41.1 ± 1.31	46.6 ± 0.7	76
150	38.1 ± 0.26	46.7 ± 1.26	49.9 ± 0.8	87
180	38.5 ± 0.65	48.1 ± 1.42	50.3 ± 0.8	86
210	36.7 ± 3.03	48.4 ± 1.71*	52.0 ± 1.	69
240	37.0 ± 1.35	47.8 ± 2.66	46.0 ± 3.	77
Number of mice	34	31	31	
60	28.5 ± 0.62	30.0 ± 0.70	29.8 ± 1	15
90	32.2 ± 0.53	33.3 ± 1.27	31.8 ± 0	42
120	30.5 ± 0.75	31.3 ± 1.12	31.8 ± 1.	03
150	31.5 ± 0.91	33.0 ± 1.31	36.1 ± 1.	15
180	33.4 ± 0.72	35.5 ± 1.41	37.2 ± 1.	12
210	35.7 ± 1.55	32.1 ± 2.30	33.6 ± 1.	35
240	35.5 ± 1.01	36.1 ± 1.51*	37.2 ± 1.	23

* Differs from control value, $P < 0.05$.

Differs from control value, $P < 0.05$.

* Differs from control value $P < 0.05$.

TABLE 1

Tumors and Precancerous Lesions Found in Sections Examined from Mice Receiving Arsenic, Germanium, Tin, and Vanadium

Group	No. of mice	All lesions		All tumors		Malignant tumors	
		No.	%	No.	%	No.	%
Controls	111	62	55.8	55	49.6	15	13.5
Arsenic	81	35*	43.0	11*	13.6	6	7.4
Germanium	103	32*	31.0	25*	24.3	7	6.8*
Tin	58	25	43.1	22	38.0	6	10.3
Vanadium	38	16	42.1	15	39.5	6	15.8
Totals	391	170	43.5	128	32.7	40	10.2

* Differs from control number, $P < 0.0005$.

* Differs from control number, $P < 0.025$.

* Differs from control number, $P < 0.05$.

Other differences from control not significant.

DISCUSSION

Arsenic, especially in its trivalent form, has been considered a carcinogen for many years (2). This idea, which appears to have had little experimental backing, has been refuted (1). The present experiments, carried out for the lifetimes of mice, indicate that at the doses given, trivalent arsenic as sodium arsenite is not toxic to carcinogenic, but actually appears to suppress spontaneous tumors in the lung.

Germanium, as sodium germanate, did not appear to be carcinogenic. There was some indication that mice receiving this element had fewer tumors (Table 1) without potentiation by type. There was no evidence in this study that the ingestion of stannous tin or tetravalent vanadium influenced the incidence of tumors. Tin dust, however, was unavoidably high in vanadium, although only low in the other metals studied, making this group of questionable significance.

According to chemical analyses, the metals given accumulated in the tissues of mice, especially arsenic, germanium, and tin. These data will be reported elsewhere (5). Although it is difficult to measure the amount of water accurately, owing to the habits of mice in wasting it, we have calculated that they ingested roughly 7 ml 100 gm body weight/day, which amount would supply approximately 35 μg arsenic/100 gm/day or about 13 mg/100 gm/year. The ingestion of 6 gm food/100 gm/day would supply an additional 2.70 μg arsenic, 1.02 μg germanium, 1.08 μg tin, and 1.42 μg vanadium per 100 gm body weight per day. The yearly intake from both food and water, on these bases, would amount to 4.8 mg arsenic, 13.5 mg germanium, 13.1 mg tin, and 18.9 mg vanadium per 100 gm body weight. These intakes are 17–20 times those measured on a 70-kg man in the cases of arsenic (3), germanium (4), and vanadium (6), and 6.4 times that of tin (7).

Experience with retrodescendant mice of this strain in three groups comprising over 400 control animals has revealed tumor incidences of 32% (8) to 40% (9). We do not believe that the animals in our study represented a strain which was resistant to tumors, as they were randomly selected litter mates distributed among the five groups. Because of the large number of animals, the control groups and those given arsenic and germanium were run concurrently; those on vanadium and tin were started a year earlier and demonstrated by other controls (5).

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A SPECTROCHEMICAL STUDY OF THE NORMAL RANGES OF CONCENTRATION OF CERTAIN TRACE METALS IN BIOLOGICAL MATERIALS[1]

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(Received for publication November 3, 1939)

It has come to be recognized that certain metals, once believed to be foreign and dangerous to living material, or to be accidental, at most, in their occurrence therein, are regular and normal constituents of the tissues and excretions of animals and men. Conclusions as to the effects of these metals on health and disease must be based, therefore, on quantitative information as to the limits of their normal concentration in biological materials, as well as to the ranges of concentration which may give rise to signs of deficiency on the one hand or of excess on the other. In the pursuit of such information considerations of convenience, specificity, and accuracy have led us to use quantitative spectrochemical methods for the estimation of a series of trace metals in various biological materials under a variety of conditions. Our methods for the determination of lead (Cholak, '35 a, '35 b), and bismuth (Cholak, '37), were recently improved and were extended so as to make possible the simultaneous determination of a number of metals in a single small sample (Cholak and Story, '38).

The data presented herein are concerned largely with the concentrations of manganese, lead, tin, aluminum, copper, and

[1] Presented before the Section of Biological Chemistry at the Ninety-eighth Meeting of the American Chemical Society at Boston, Mass., September 11-15, 1939.

silver which occurred in duplicates of the composite 24-hour food samples, in the excreta of apparently normal healthy human adults, and in the tissues of selected persons whose activities had given them no unusual or occupational exposure to these metals. A considerable volume of information on the lead content of individual food items, natural waters, and samples of soil from various portions of the earth's surface has accumulated since we recorded our earlier results and reviewed the work of other investigators (Kehoe et al., '33); this is presented herein. Reference to the numerous articles concerning the trace metals from other laboratories has of necessity been reserved for a separate review.

ANALYTICAL TECHNIQUE

Samples were prepared for analysis by procedures outlined previously (Cholak, '35 a, '35 b, '37; Cholak and Story, '38). Suitable quantities of solutions of the ashed materials, the inorganic salt compositions of which were made to conform to the standards by the method of excess (Cholak, '35 b, '37; Cholak and Story, '38), were placed in craters of purified graphite electrodes and their arc spectra were photographed while a five-step logarithmic sector was being rotated before the slit of the spectrograph (Cholak and Story, '38). The method of evaluating the spectral lines was similar to that of Strock ('36) except that quadrics were substituted for densities in evaluating very weak lines (Cholak and Story, '38). The spectral region between 2600 A and 3500 A, in which the most persistent lines of a large number of the metals occur, was photographed. Although limited in application thus far to six metals, the method as applied to this region can be extended to include others, especially iron, thallium, cadmium and nickel. Zinc, on the other hand, cannot satisfactorily be dealt with simultaneously, since its most persistent lines at 2302 A and 3345 A lack sufficient sensitivity for the detection of the small amounts of zinc normally present and are also masked by the sodium line at 2302 A and the calcium line at 3345.5 A. The detection and determination of this metal are dependent,

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therefore, on spectrograms made by a special technique in the extreme ultraviolet region (Zn line at 2138.5 A), which lacks suitably persistent lines of other metals and for which specially sensitized plates must be used. In the case of manganese, included since our latest publication (Cholak and Story, '38), the line at 2801 A was employed in conjunction with the chromium line at 2825 A as internal standard. The latter metal was added to the mixed internal standard, which then consisted of 5 mg. of bismuth, 100 mg. of cobalt and 10 mg. of chromium per 100 ml. of solution (Cholak and Story, '38).

The quantitative sensitivity of the technique used in these analyses was further increased by concentrating the solutions of the ashed materials to a point beyond that of previous practice (Cholak and Story, '38). The volumes of the final solutions of tissues and whole blood were adjusted so that each cubic centimeter was equivalent to 2 gm. of the original material, while in the case of blood plasma each cubic centimeter was equivalent to 3 ml. of original plasma. Such procedures permitted the detection of 0.005 mg. of metal per 100 gm. of fresh tissue or blood, and 0.003 mg. of metal per 100 ml. of plasma.

RESULTS

The results obtained on normal human tissues are recorded in table 1. With the exception of the tissues of one male whose occupational history was carefully checked, those examined were from females who had never been employed in industry. This choice of material has limited the number of available samples and has prevented the establishment of statistically stable mean values for the concentration of metals in the various tissues, but the arithmetical means are expected to fall near the eventual stable values.

Table 2 gives the data on the concentrations of the metals in the urine of several widely scattered groups of normal men, including thirty-four Frenchmen, thirty Mexican Indians and thirty Americans. The results with respect to lead on a group of thirteen Germans are also shown. The urine (24 hour specimen)

TABLE 1

The concentration levels of trace metals in normal human tissues (Americans, arithmetical mean values)

Tissue	Milligrams of metal per 100 gm. of wet tissue					
	Mn	Pb	Sn	Al	Cu	Ag
Kidney	0.060	0.027	0.020	0.042	0.166	0.00
Heart	0.032	0.018	0.022	0.026	0.150	0.00
Brain	0.030	0.013	0.00	0.004	0.400	0.003
Liver	0.203	0.130	0.060	0.160	0.215	0.005
Spleen	0.022	0.030	0.022	0.100	0.085	0.00
Lung	0.022	0.029	0.015	5.01	0.110	0.004
Muscle	0.050*	0.010	0.011	0.015	0.125	0.00
Long bone	0.380*	1.88	0.080	0.500	3.300	0.07
Rib bone	0.270*	0.470	0.050	0.240	0.110	0.01
Stomach	0.030*	0.022	0.050	0.073	0.192	0.00
Intestines	0.085	0.025	0.016	0.097	0.130	0.002

* Results of single analyses.

TABLE 2

The concentrations of trace metals in normal urine expressed as the mean, their probable errors, and their standard deviations

Metal	Milligrams of metal per liter of urine				
	Frenchmen	Mexicans	Americans	Germans	All groups
Mn		0.012 ± 0.001 ± 0.001	0.010 ± 0.[illegible] ± 0.[illegible]		0.01[illegible] ± [illegible]
Pb	0.020 ± 0.02 ± 0.014	0.022 ± 0.002 ± 0.017	0.029 ± 0.002 ± 0.016	0.027 ± 0.002 ± 0.012	0.027 ± 0.001 ± 0.014
Sn	0.10 ± 0.00 ± 0.00	0.001 ± 0.001 ± 0.007	0.008 ± 0.002 ± 0.013		0.011 ± 0.001 ± 0.010
Al	0.114 ± 0.006 ± 0.048	0.051 ± 0.004 ± 0.031	0.072 ± 0.003 ± 0.02[illegible]		0.078 ± 0.002 ± 0.032
Cu	0.034 ± 0.002 ± 0.026	0.039 ± 0.003 ± 0.024	0.028 ± 0.002 ± 0.019		0.034 ± 0.002 ± 0.0[illegible]
Ag	0.00	0.00	0.00		0.00
No. of samples	34	30	30	13	9[illegible]*

* Total in case of lead 107, in case of manganese 90.

mens) of a healthy experimental subject was examined for 28 consecutive days and the results, expressed in milligrams of metal per liter of urine, are grouped in accordance with their frequencies of occurrence, in table 3, together with the calculated means, the probable errors of the means, and the standard deviations from the means.

Blood samples from thirty normal Americans and thirty normal Mexicans Indians provided the results in table 4, in

TABLE 3

The concentrations of trace metals in successive daily urine samples of a normal adult American

Milligrams per liter	Number of occurrences of metal concentrations indicated					
	Mn	Pb	Sn	Al	Cu	Ag
0.008–0.009	28		2	1	2	28
0.010–0.019		1	17	1	5	
0.020–0.029		8	3	3	7	
0.030–0.039		11	1	5	3	
0.040–0.049		4		1	5	
0.050–0.059		1		1	1	
0.060–0.069				5	2	
0.070–0.079				1	2	
0.080–0.089				1		
0.090–0.099				2		
0.100–0.109						
0.110–0.119				1	1	
Number of samples	28	28	28	28	28	28
Mean	0.01	0.024	0.014	0.052	0.037	
Probable error	± ?	±0.001	±0.001	±0.003	±0.002	
Standard deviation	± ?	±0.008	±0.007	±0.025	±0.014	

which the mean concentrations of the metals in whole blood are shown. More detailed data obtained on twelve consecutive weekly samples of the blood of a normal subject appear in table 5. Blood samples of the American group were obtained in duplicate by dividing a 30 ml. sample of blood at the time of its withdrawal. One portion was used to determine the concentrations of the metals in the whole blood (table 4), while the other, after treatment with an anticoagulant (purified sodium citrate),[2] was centrifuged at once and the separated plasma was analyzed. The quantities of metals in the plasma of 100 gm. of whole blood were calculated in each case, and those in the formed elements were taken as the difference

TABLE 4

The concentrations of trace metals in normal blood

Whole blood, per 100 gm.	Mn	Pb	Sn	Al	Cu	Ag
Mexicans:						
Mean	0.018	0.021	0.010	0.012	0.126	Trace
Probable error	±0.001	±0.0005	±0.0015	±0.001	±0.002	
Standard deviation	±0.010	±0.004	±0.012	±0.010	±0.018	
Americans:						
Mean	0.012	0.027	0.014	0.014	0.193	Trace
Probable error	±0.001	±0.0006	±0.0015	±0.002	±0.002	
Standard deviation	±0.006	±0.005	±0.012	±0.012	±0.013	
Whole groups:						
Mean	0.015	0.025	0.012	0.013	0.111	Trace
Probable error	±0.001	±0.0004	±0.0009	±0.001	±0.002	
Standard deviation	±0.008	±0.005	±0.010	±0.011	±0.020	
Highest value in single [illegible][1]						
Whole blood:						
Mean	0.012	0.027	0.011	0.014	0.103	Trace
Probable error	±0.001	±0.0006	±0.0015	±0.002	±0.002	
Standard deviation	±0.006	±0.005	±0.012	±0.012	±0.013	
Plasma:						
Mean	0.004	0.0015	0.002	0.024	0.093	
Probable error	±0.0005	±0.0002	±0.0002	±0.003	±0.0015	
Standard deviation	±0.003	±0.0013	±0.0014	±0.012	±0.012	
Cells:						
Mean	0.008	0.024	0.011	0.003	0.159	
Probable error	±0.001	±0.0004	±0.001	±0.0003	±0.002	
Standard deviation	±0.006	±0.006	±0.008	±0.002	±0.016	

[1] Results based on 100 gm. of whole blood.

between plasma and whole blood. The calculated mean values for the whole blood, cells, and plasma of the group are listed in table 4. The histogram in figure 1 details the spread in the results obtained for the copper and lead content of the plasma.

[2] In order to check the possible precipitating effect of sodium citrate on the distribution of lead in the blood, a highly potent heparin preparation was used as the anticoagulant in a number of blood samples, with results similar to those in samples treated with sodium citrate.

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While the consecutive urine samples referred to in table 3 were being obtained, the corresponding feces and duplicate samples of the food and beverages taken by the subject were collected for analysis in 24-hour samples. The results are expressed in table 6 as mean daily intake and output.

Additional data concerning lead are given in tables 7 to 10.

TABLE 6

Comparison of the mean daily quantities of trace metals in successive samples of the feces and food of a normal adult American

	Milligrams per 24 hours, found.					
	Mn	Fe	Cu	Al	Pb	Ag
Food						
Mean	4.28	0.290	17.14	30.13	2.32	0.044
Probable error	±0.42	±0.027	± 1.30	± 7.20	±0.28	±0.016
Standard deviation	±2.39	±0.213	±10.17	±31.97	±2.21	±0.127
Feces						
Mean	3.69	0.320	23.84	41.02	1.96	0.058
Probable error	±0.30	±0.019	± 1.30	± 6.12	±0.18	±0.005
Standard deviation	±2.30	±0.111	±10.28	± 40.80	±1.23	±0.032

DISCUSSION

The concentrations of these metals in normal tissues, blood and urine are seen to be low, and, excluding copper and silver, are of the same order of magnitude. The concentration of copper is seen to be sevenfold that of the other metals in practically all tissues, while in the case of silver, which is irregular in occurrence, only minute amounts are encountered. The pattern of the distribution of these metals in the human organism is roughly similar in that their highest concentrations generally occur, in decreasing order of magnitude, in bones, liver, kidney and spleen. The most striking exceptions are the concentration of copper in the brain, which ranks next to that in the liver, and the concentration of aluminum in the lung, which is higher than that in any other tissue, largely, no doubt, because of the inhalation of dust. Since aluminum is the most widely distributed metal on the earth's surface, inhaled dusts, especially from streets, may be expected to be rich in aluminum. This omnipresence of aluminum may exert

TABLE 5

The concentration of trace metals in successive weekly blood samples of a normal adult American

Milligrams per liter.	Found in the quantities of the specimen indicated.						
	Mn	Fe	Sn	Al	Ag	Milligrams per liter.	Cu
0.000–0.00[illegible]			0	4	18	0.070–0.079	2
0.010–0.0[illegible]	5		3	5		0.080–0.089	
0.0[illegible]–0.0[illegible]	4		4	2		0.090–0.099	4
0.0[illegible]–0.0[illegible]	4		1	1		0.100–0.109	1
0.020–0.029	4	1	1	1		0.110–0.119	2
0.0[illegible]–0.0[illegible]		4		1		0.120–0.129	1
0.0[illegible]–0.0[illegible]		1		1		0.130–0.139	2
0.0[illegible]–0.0[illegible]	1	3		1			
0.0[illegible]–0.0[illegible]				1			
Number of samples	18	11	18	18	18		18
Mean	0.015	0.030	0.008	0.015	0.00[illegible]		0.108
Probable error of mean	±0.001	±0.001	±0.000	±0.002	?		±0.003
Standard deviation	±0.006	±0.007	±0.000	±0.012	?		±0.018

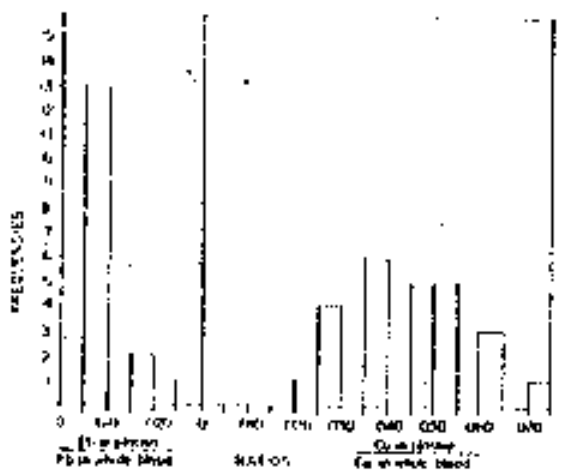

Fig. 1 Histogram showing the variation in the relationship between the plasma and the whole blood of normal human adults, with respect to their concentrations of lead and copper.

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a serious influence upon the accuracy of determinations of this metal, since dust may be carried on the shoes and clothing of workers into the most ideal laboratory, the air of which is filtered. Our consistently low and substantially uniform results indicate that such contamination was kept at a minimum. In this connection it is to be remembered that the samples of blood plasma were concentrated one-third more highly than were the whole blood samples, and thus they gave somewhat higher results than the latter, on account of the increased analytical accuracy obtained thereby and not because of contamination.

Manganese, lead, aluminum and copper were regularly present in all samples of human tissue and urine, while tin was found in about 80% and silver in only 10% of the samples. Although all metals but silver were frequently present in blood, there were decided differences in the distributions of these metals between the cells and plasma. Thus, manganese, lead and tin were located principally in the cells, aluminum in the plasma, and copper fairly evenly divided between the two, the cells usually containing slightly larger quantities.

All six metals occurred regularly in the feces and in composite food samples, and in concentrations considerably greater than those found in the tissues and the urine. In the case of each metal, the actual quantities found in the series of 24 hour samples of feces were of the same order of magnitude as those in the 24 hour samples of food, a fact which, in view of the low concentrations in the tissues and the urine, not only demonstrates that each of these metals is eliminated almost completely by the alimentary tract, but also suggests that they are but scantily absorbed by the alimentary tissues.

The detailed sources from which these metals are derived require further elucidation. It is well known that certain foods are high in manganese and copper, and that tin, aluminum, and silver in the dietary are derived to a large degree from the use of food containers, cooking utensils, and tableware, made of these metals. Only in the case of lead, however, are our data sufficiently numerous and varied to provide a

TABLE 4

The natural lead content of soil, water, and vegetation

MATERIAL	SOURCE	RANGE	NUMBER OF SAMPLES
		p.p.m.	
Soil (dry)	Northeastern U.S.A.	7.0 – 55.7	10[1]
Soil (dry)	State of Mexico, Mexico	0.70 – 8.0	3
Soil (dry)	Yucatan	0.90 – 25.0	39
Soil (dry)	Suriname	1.20 – 3.0	2
Soil (dry)	River bottom in Suriname	4.8	1
Tea leaves (dry)	Ceylon	0.02	1
Fruit of tree (wet)	Yucatan	0.13 – 0.30	2
Foliage (wet)	"	0.25 – 0.60	3
Bark (dry)	"	0.04 – 0.40	18
Root (dry)	"	0.05	1
Latex (wet)	"	0.04 – 0.08	36
Bark (dry)	Suriname	0.04 – 0.25	6
Latex (wet)	"	0.02 – 0.03	4
Cocoa beans (dried husks)	Trinidad	0.40 – 1.60	14
Cocoa beans (dried nibs)	"	0.03 – 0.35	13
Peas (green)	State of Mexico	0.03	1
Peas (green pods)	" " "	0.05	1
Beans (green)	" " "	0.15 – 0.26	2
Beans (green pods)	" " "	0.04	1
Cherries (green)	" " "	0.05	1
Apples (green, unsprayed)	" " "	0.12	1
Pears (green, unsprayed)	" " "	0.18	1
Corn (green stalk)	" " "	0.05 – 0.11	3
Corn (green husk)	" " "	0.26	1
Corn (green on cob)	" " "	0.03 – 0.21	3
Wheat (green)	" " "	0.27	1
Radishes (green)	" " "	0.28	1
Sea water	Caribbean Sea	less than 0.02[2]	2
Sea water	Pacific Ocean	less than 0.02[2]	2
Sea water	Atlantic Ocean	0.003–0.005	2
River water	Suriname	0.005	1
Pond water	"	0.004 – 0.02	2
Well water	Mexico	0.009	1
Stream water	"	0.009	1
Well water	U.S.A.	0.02 – 0.03	2

[1] Data of Jones and Hatch, Soil Science, vol. 44, p. 27 (1937).
[2] Limit of sensitivity of analytical method then in use.

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fairly comprehensive picture of its origin in the diet. The wide distribution of lead is clearly indicated in tables 7 to 10. Its presence in the soil and in the vegetation growing therefrom, is shown in table 7. In our experience, as illustrated in these data, the natural lead content of vegetation rarely exceeds a small fraction of a part per million, but there is always some small quantity, whether the plant grows in the surface or is deeply rooted in the earth. There is a considerable variation among plants and in different parts of the same plant, with respect to lead concentration, but probably not to the extent indicated by the data, since certain of the samples could not

TABLE 8

The natural lead content of coral (Florida Keys)

[illegible]	[illegible]
0.00 – 4.99	1
5.00 – 9.99	7
10.00 – 14.99	11
15.00 – 19.99	4
20.00 – 24.99	1
25.00 – 29.99	
30.00	1
Total	25

TABLE 9

The lead content of bone (Jefferson). No occupational lead exposure

[illegible]	[illegible]	
	Male	Female
6	1.02	1.14
207	1.11	
51	0.17	
64		0.80
70	0.39	3.50
75	0.60	2.39
95	0.56	1.36

be so handled as entirely to exclude the possibility of surface contamination with soil or atmospheric dust, although every effort was made to do so.

The occurrence of lead in natural waters is also indicated in table 7. The results on sea water bear a significant relationship to those obtained on a series of coral skeletons, as shown in table 8. The latter samples were obtained for us by R. L. Clary of Princeton University (whose assistance we gratefully acknowledge), from the Tortugas Laboratory of the Carnegie Institution, with such precautions in packing as would prevent surface contamination. These analyses were made because of speculation as to the possibility of the development of substantially lead free soils on a coral island. Since it is apparent

that there is a selective absorption of lead by the coral skeleton, it is scarcely to be expected that a soil founded upon such material would support the growth of lead-free plants and animals.

TABLE 10

[illegible]

[illegible]	Lead	No. of samples
	p.p.m.	
Wheat bread	0.02–0.06	6
Bran flakes	0.11–0.13	2
Crackers and pretzels	0.21–0.25	3
Spaghetti (prepared for eating)	0.06–0.21	3
Corn (dry)	0.27	1
Cornstarch	0.75–1.83	4
Corn syrup	0.21–0.39	2
Cocoa (29 brands)	0.4–11.5	29
Tea (dried leaves) Chinese	43.2	1
Cabbage	0.10–0.24	4
Fruits (raw and cooked)	traces–1.00	10
Beef liver (fresh)	0.29–0.40	3
Meat (cooked)	traces–0.63	9
Meat (ground, cooked)	0.13–0.18	3
Sausages (cooked)	0.16–1.01	4
Eggs (raw and cooked)	traces–0.12	6
Coffee (prepared for use)	0.01–0.03	3
Milk	0.02–0.04	3
Beer	0.01–0.09	21
Grape juice	0.04–0.49	7
Wine (domestic and imported)	0.05–1.51	16
Water	0.02–0.05	10
Water	0.37–0.92 [1]	3

[1] Obtained from building in which water was stored or coursed in pipes for some days.

As shown by the standard deviation of the mean value (table 6) the freely chosen daily diet of a normal healthy adult shows considerable variation in its lead content. It is not to be assumed that all such lead is of natural origin, although the results on commonly available foods and beverages, as listed in table 10, show that such is the origin of a considerable proportion of it. As we have pointed out elsewhere (Kehoe et al., '33), the increment of lead content above that which is a

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natural constituent is due to contamination from a great variety of sources.

The essential equivalence of the mean daily intake and output of lead, as previously referred to in commenting on the results for the entire group of six metals, is worthy of special note in connection with the data of table 9, which fail to reveal any relationship between age and lead concentration in the skeletal tissues. These data are obviously too few to give direct and definite evidence against an indefinitely progressive accumulation of lead in the body in response to the customary regular intake of lead over a prolonged period, but they are presented because of the care taken against the inclusion of abnormal material among the samples.

SUMMARY

A quantitative spectrographic method of high sensitivity and precision has been employed for the simultaneous determination of lead, manganese, tin, aluminum, copper and silver in normal biological material.

1. Lead, manganese, copper and aluminum were present in all materials examined; tin was present in about 80% and silver in 10 to 20% of the samples.

2. The mean concentrations of these metals in a liter of normal urine has been found to be below 0.01 for manganese and 0.078, 0.034, 0.027, 0.011, and 0.00 mg. respectively for aluminum, copper, lead, tin and silver.

3. The mean concentrations of the metals in 100 gm. of normal whole blood are 0.114, 0.025, 0.015, 0.013, 0.012, and 0.00 mg. respectively for copper, lead, manganese, aluminum, tin and silver.

4. Practically all of the manganese, lead and tin is contained in the formed elements of the blood; aluminum is found almost entirely in the plasma, while copper is almost evenly divided between the two, the formed elements usually containing a slightly higher concentration.

5. The concentrations of these metals in consecutive daily or weekly samples of urine and blood from the same individual are not constant but vary from sample to sample.

6. The daily output of this group of metals in the feces is practically equivalent to their daily intake in the diet.

7. The wide distribution of lead is indicated by data obtained on a large number of natural materials.

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Right-hand agenesia of the not prematurely new-born child.
Discussion of the possible aetiological role of the absorption by the mother, during the second month of pregnancy, of a taenifuge with a metallic tin base, associated with an absorption of pyridoxine.

by A. Notter, J.M. Robert and G. Boudenes.

We had the opportunity of observing the following case, and it seemed appropriate to us to relate it at a time where Thalidomide has revealed the teratogenous influence of certain treatments.

Mrs. D..., second pregnancy, 28 years of age. A precise examination has revealed no malformative antecedent in her family or in her husband's. First child completely normal (daughter).
Spontaneous non-premature confinement on May 24 1963: a living and robust boy with a weight of 2,950 g, revealing a complete agenesia of the right hand. It is difficult to assess the anatomical state of the carpus; the ossification spots do not appear on the radio, but they never do immediately after birth. The inferior extremity of the ulna appears to be definitely hypoplasic. The inferior extremity of the radius is normal. Besides these observations, no other anomaly is revealed by the examination. Normal cardiac auscultation.
During pregnancythere was no clinically identifiable infectious episode nor irradiation. At the beginning of her pregnancy, the mother has established the existence of a taenia saginata in her, and requested her physician to treat that parasitism while she was in Algeria. From October 28 to November 2, 1962 (5 days), she took

3 tablets per day of a taenifuge containing pure tin oxyde: 0.06 g and pure metallic tin 0.06 g per tablet, in association with one tablet per day of pyridoxine at 250 mg, from October 28 until October 31, 1962.

The mother of this child afflicted with a malformation was very precise in her answers. Therefore we are certain that no aetiological factor, presently recognized as a possible cause for dysplasia has existed in this case. Therefore, besides an always possible new mutation, but which would be impossible to prove (except perhaps in the future descent of this ectromelic boy), and a hypothetical "amniotic bridle" of which the mechanism is rarely put forward nowadays (for a lack of obvious demonstration), we were brought to discussing the teratogenic role of the absorbed antiparasitic drug, although our bibliographic research has not shown us anything similar as far as experimental observations and clinical cases are concerned. This taenifuge, of which several very similar versions have been presented by different laboratories, contains tin oxyde and metallic tin, both chemically pure. But, theoretically, these substances are not absorbed by the body, but they are rejected with the faeces after having acted on the parasite in the intestinal light. In this effect they are to be distinguished from organic derivatives of tin: iodized or ethylic derivatives which are perfectly absorbed, and which were at the origin of the Stalinon case.

This product is specialized since 1934. Hundreds of thousands of samples were sold and new analytical tests were carried out in 1952 on the occasion of a request of visa renewal. It is therefore unlikely that the formula of the specialty was altered and that this alteration has made the tin absorbable by the intestinal mucous membrane.

If this were the case, it should also be admitted that the placental barrier was crossed, a hypothesis we cannot prove.

In reality, we are inclined to admit a simultaneous and perfect association with pyrodoxine which intervenes strongly in numerous metabolic transformations, namely as a transamination and decarboxylation agent. Is not the vitamin B6 susceptible of having provoked a transformation of a mineral derivative of tin into an absorbable organic derivative, within the intestine ?

A last question to be solved is the date at which the product was absorbed: it is situated at the end of the second and the beginning of the third month of pregnancy. As far as the Thalidomide is concerned, Lenz sets the dangerous period between the 37th and the 50th day after the last menstruation. About the 60th day we are at the extreme limit of the "teratogenous period", particularly in cases with a transversal ectromelia. But even in this field, certain doubts remain, which account for our extreme prudence in relating this observation which, however, stresses the possible danger of any treatment given during the first three months of pregnancy.

Obligatory declaration of all malformations is desirable, in order that a genetician might carry out an investigation that is much easier at the moment of birth than in retrospection. Thus, it would be easier to compare suspect cases of drug etiology and to carry out experimentations with full knowledge of the facts.

Discussion

Mr. Brochier insists on the fact that such observations should be interpreted very cautiously. Their interest resides in the possibility of gathering them in a file of temporary facts.

Agénésie de la main droite chez un nouveau-né à terme.

Discussion du rôle étiologique possible de l'absorption par la mère, au deuxième mois de la gestation, d'un tranquilisant à base d'étain métallique associé à la Pyridoxine.

par MM. A. Netter, J. M. Robert et G. Boudenes.

Nous avons eu l'occasion d'observer le cas suivant qu'il nous a paru bon de rapporter à l'époque où la Thalidomide a mis à l'ordre du jour l'influence tératogène de certaines médications.

Mme D... IIe parc, 28 ans. Un interrogatoire précis ne révèle aucun antécédent malformatif dans sa famille ni dans celle de son mari. 1er enfant entièrement normal (fille).

Accouchement spontané à terme le 24 mai 1963 : garçon vivant et vigoureux pesant 2 950 g, présentant une aréflexie complète de la main droite. Il est difficile de se rendre compte de l'état anatomique du carpe ; les points d'ossification sont absents sur le radius mais ils le sont généralement à la naissance. Quant à l'extrémité inférieure du cubitus elle apparaît assez nettement hypoplasique. L'extrémité inférieure du radius est normale. Le reste de l'examen ne montre pas d'anomalie. Hospitalisation cardiaque normale.

Il n'y a eu durant la grossesse ni épisode infectieux cliniquement décelable ni irradiation. Au début de la grossesse la mère a consulté chez elle l'existence d'un mycose vaginale et a demandé à son médecin de traiter ce parasitisme alors qu'elle se trouvait en Algérie, d'où la prise du 28 octobre au 2 novembre 1962 (5 jours) de 3 comprimés par jour d'un tranquilisant contenant oxyde d'étain pur 0,06 et étain métallique pur 0,06 par comprimé en association avec, du 28 octobre au 31 octobre 1962, un comprimé par jour de Pyridoxine à 250 mg.

La mère de cet enfant malformé ayant pu être extrêmement précise dans ses réponses, nous sommes assurés qu'aucun facteur étiologique actuellement reconnu comme cause éventuelle de dysplasie n'a existé dans ce cas. C'est pourquoi, en dehors : d'une mutation nouvelle, toujours possible, mais impossible à prouver (sauf peut-être dans la future descendance de ce garçon actuellement), d'une hypothèse « bizarre » mais intéressante dont le mécanisme est bien mal connu de nos jours (faute de démonstration patente), nous avons été amenés à discuter le rôle tératogène de la médication antipaludique absorbée, bien que nos recherches bibliographiques tant en ce qui concerne les observations expérimentales que les cas cliniques ne nous aient rien révélé de semblable.

Ce tranquilisant, dont il existe d'ailleurs plusieurs versions assez proches présentées par différents laboratoires contient de l'oxyde d'étain et de l'étain métallique chimiquement purs. Or, théoriquement ces corps ne sont pas absorbés et, agissant sur le parasite dans la lumière intestinale sont ensuite rejetés dans les fèces. Ils s'opposent en cela aux dérivés organiques de l'étain : dérivés iodés ou éthylés, parfaitement absorbés eux, et qui furent à l'origine de l'affaire du Stalinon.

Le produit est spécialisé depuis 1934. Des centaines de milliers d'échantillons en auraient été vendus et de nouveaux tests analytiques ont été réalisés en 1952, lors d'une demande de renouvellement de visas. Il est donc peu probable que la formule de la spécialité ait été altérée et que cette altération ait rendu l'étain absorbable par la muqueuse intestinale. Dans cette opportunité, il faudrait encore admettre que la barrière placentaire ait été franchissable, ce que nous ne saurions prouver.

En réalité, nous soupçonnons plutôt l'*association parfaitement simultanée avec la*

Pyridoxine qui intervient puissamment dans de nombreuses transformations métaboliques, notamment comme agent de transamination et de décarboxylation. La vitamine B6 n'est-elle pas susceptible d'avoir provoqué dans l'intestin une transformation d'un dérivé minéral de l'étain en dérivé organique absorbable ?

Une dernière question à résoudre est celle de la date à laquelle le produit a été absorbé : elle se situe à la fin du deuxième mois de gestation et au début du troisième. Pour la Thalidomide, Lenz situe la période dangereuse entre le 37e et le 50e jour après les dernières règles. Autour du 60e jour nous nous trouvons à l'extrême limite de la « période tératogène », surtout dans le cas d'une extranéité transversale. Mais, dans ce domaine aussi, des incertitudes demeurent d'où notre extrême prudence dans le rapport de cette observation qui met cependant l'accent sur le danger possible de toute médication donnée dans le premier trimestre de la gestation.

Il faut souhaiter la déclaration obligatoire des malformations afin de confier à un généticien l'enquête étiologique beaucoup plus facile au moment de la naissance que rétrospectivement. Ainsi seraient plus facilement colligés les cas suspects d'étiologie médicamenteuse et l'expérimentation entreprise à bon escient.

Discussion.

M. Broelder insiste sur la prudence avec laquelle il faudrait interpréter de telles observations. Leur intérêt est de les rassembler dans un dossier de faits en attente.

Cétoïrienne pour cardiopathie.

par MM. H. Rigeaud, E. Neumann et J. C. Couturier.

Mme F... 36 ans, IIIe parc. Malade ayant eu une connaissance de migraine, au résultat indécise et qui entre dans le Service pour repos et thérapeutique avant le terme. Les antécédents personnels sont chargés avec, à l'âge de 11 ans, une cystite dont elle n'a gardé aucune séquelle quant à la miction ;

— à l'âge de 13 ans, hypertrophie cardiaque [illegible] par le spécialiste ;

— à 14 ans : [illegible] ;

— constipation à l'âge de 17 ans d'un rétrécissement mitral.

Les antécédents obstétricaux sont les suivants :

— en 1946 : grossesse bien supportée. Accouchement à 8 mois d'un garçon de 1 700 g (forceps) ;

— en 1947 : grossesse bien supportée. Accouchement à terme d'un garçon de 2 500 g (forceps) ;

— en 1954 : fausse couche spontanée à 5 mois suivie d'une ovariectomie et salpingectomie droite.

Entre temps la malade a présenté de nombreuses crises de rhumatisme articulaire aigu traitées par antibiothérapie et corticothérapie.

En 1959 : nouvelle fausse couche spontanée de 2 mois.

La grossesse actuelle a évoluée mal supportée (dernières règles : 22 août ; Accouchement prévu pour le 31 mai 1963). Au 4e mois, nausées de l'aube [illegible] et intoxication d'un traitement hormonal. Par ailleurs, apparition progressive d'une dyspnée qui va en s'aggravant. La malade est revue à 2 mois 1/2 de grossesse, très dyspnéique ; elle est alors mouillée au Ve Fleurent qui, devant l'existence d'un rétrécissement mitral serré, pose l'indication de commissurotomie immédiate. Celle-ci est pratiquée à 5 mois 1/2 de grossesse ; l'intervention

Scripta Medicina [illegible]

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Z. PELIKAN, K. HALACKA, E. ČERNY

Acute Toxic Effect of Stannous Chloride on White Mice

This short paper deals with the clinical state and pathological findings, observed in white mice following the application of tin, in the form of $SnCl_2$, by means of a stomach tube. It is one a series of experiments dealing with the problem "Toxicity of tin", the results and dynamics of which will be published later in a complete work. This problem of late, has been the subject of much discussion not only from the standpoint of the presence of tin in food as a natural trace element, but also with regard to its presence in food as a foreign body and, therefore, the possibility of its possible toxic effect on the organism. Views on this problem vary considerably.

In most papers published to date on the toxicity of tin, especially of stannous chloride, the tin has been administered to experimental animals i. v., i. m. or s. c. (2, 5, 7, 11) and comparatively little has been written about its intake through the digestive tract (13). In the literature so far available, we have not met with the application of Stannous chloride by means of a stomach tube. We have, therefore, chosen this method for our experiments.

METHOD

Stannous chloride cryst. c.p. (Pb 0.01%, prepared in Poland) was applied in a dose of 1000 mg./kg. body weight to white mice. This dose is about five times higher than the LD_{50} determined by us in white mice (LD_{50} per os = 213 mg./kg. b.w., span 158.6–283.8 mg./kg. b.w.). LD_{50} as determined by us was calculated by *Polzer* by the "Probit method" (10). This high dose was chosen with the purpose of ascertaining the type of toxic damage and to determine the main signs in the animal organism. As $SnCl_2$ is only soluble in an acid medium, we chose for its solution pure filtrated lemon juice, which [illegible] Vienna is the best and most physiological medium for the animal organism. The taste of this solution was improved by 1 g of castor sugar for each 2 ml of the solution.

White mice (of "H" breed – Czechoslovak standard breed; Corp. "VÚTAZ") of average weight 25 g. of both sexes (male and female equally divided), were used as experimental animals. Each mice from the experimental group was given one dose of $SnCl_2$ [illegible] ml of lemon juice and each mouse of the control group was given 0.1 ml pure lemon juice only. The administration was carried out by means of a stomach tube. On the day of the administration the mice were left without water to avoid the possible separation of the $SnCl_2$ from the solution by the action of water in the contents of [illegible]

For the experiment were always used 6 animals (3 male and 3 female) in the "Sn... group" and 6 animals in the "control group". These experiments were repeated three times on the same number of animals. Both the clinical and the macroscopic results at autopsy (dissection) were each time identical. The viscera from the last experiment were subjected to histological examination.

The clinical state and its changes were systematically followed and determined at two time-intervals, 4 hours and 8 hours, after the administration. Eight hours after the administration the experimental animals were killed (decapitated and dissected). The viscera were immediately fixed in 10% solution of formalin for a period of 48 hours, then embedded in paraffin and after treatment stained with haematoxylin and eosin.

RESULTS

Clinical findings

Four hours after the application the mice in the "$SnCl_2$ group" were very weak, lying on their sides or on their bellies, there was debility of limbs, laboured periodic respiration developed, the heads hung down and the eyes were closed; the coats bristled, the acral parts (tails, paws) were cyanotic. The mice did not respond to sound stimuli and only slightly to mechanical stimuli. In the course of a further 4 hours this state became emphasized. There was complete debility, depression of physiological functions; reactibility and respiration rate decreased.

Mice in the "control group" showed no changes in the clinical findings.

Macroscopic results on autopsy

The results were practically identical in all animals of the "$SnCl_2$ group", and may be characterised thus: The intestines were expanded; serosa considerably congested; in most animals there were single petechiae on the stomach serosa; spastic and dilated sections alternated along the small intestine; livers were darker red, than normal considerably congested, the tissue was homogeneous; hyperemic lungs were dark red. No considerable deviations from standard were found on other organs.

In the "control group" no macroscopic changes were found (only in one mouse the liver was marbled in appearance on the surface).

Histological findings

a) In the animals of the $SnCl_2$ group

The liver

The structure of the liver was preserved, but in the tissue there were dispersed non-systematic, roughly circular necroses, in some of which were rather numerous neutrophils and a few large mononuclears of the histiocytic type. Some necroses were nodular in appearance but in structure were not everywhere identical. In approximately one third of the animals foci of complete coagulation necroses were found subcapsularly (fig. 1). The coagulation necroses were approximately cuneiform and in area roughly the size of the lobule. Disintegrating leucocytes were found in some of them.

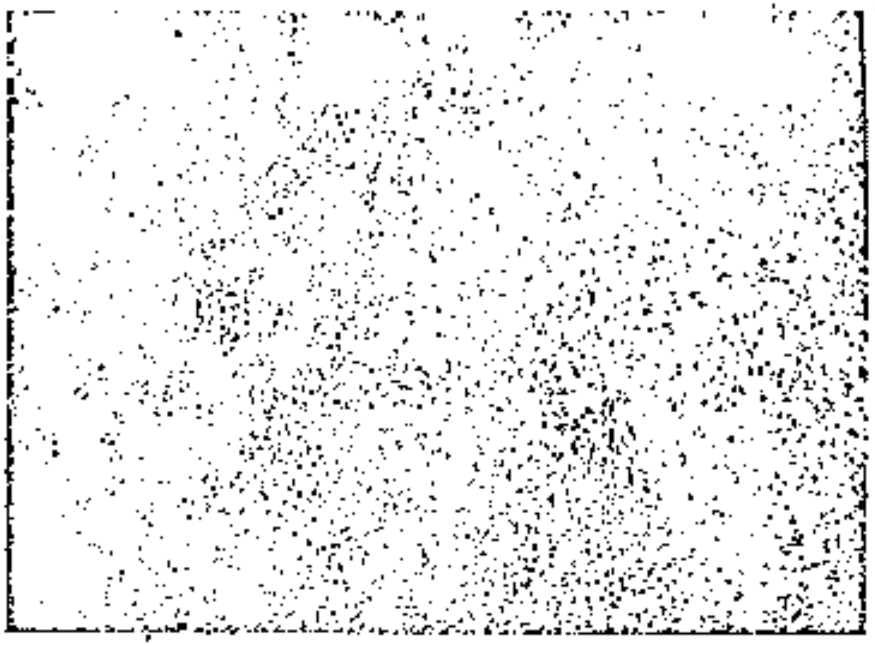

Fig. 1. Focus of necrotic liver parenchyma with reactive and regressive changes.

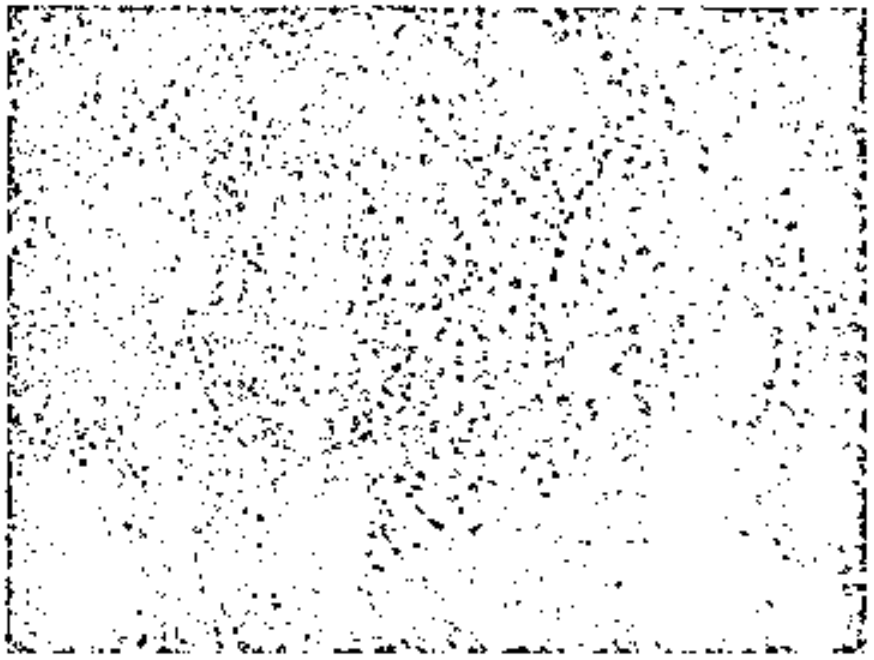

Fig. 2. Focal reactive necrosis in splenic tissue

In other parts of the liver parenchyma the cells had the parenchymatous cloudy swelling or showed vacuolar degeneration and, in some of them, fatty degeneration prevailed. This degeneration of the cells changed quantitatively in each preparation. In approximately one half of the experimental animals, mainly mixed obturating thrombi were found in the smaller intrahepatic branches of v. portae.

There were no pathological changes visible in the other structure, namely, portal fields, liver sinusoids and reticuloendothelial system.

The spleen

In the red pulp there were small nodular necroses, similar to those in the liver. In these necroses there were numerous partially disintegrated leucocytes (Fig. 2). In the follicuti there were mononuclear necroses containing single leucocytes. Some of the necroses in the pulp had disintegrated. In one animal numerous histiocytes were found in the neigbourhood of the necroses. In single cases fresh obturating thrombi were found in the arteriolar lumens. RES of spleen was mostly hyperplastic, the sinusoids were greatly congested.

Myocardium

In one mouse small necroses were found in the myocardium, with some leuco-lymphocytar infiltrates, and in another mouse small focal mononuclear infiltrates were also found. These findings can be assessed as inflammatory. Other organs were without pathological changes.

b) No changes were found on the organs of the "control group".

DISCUSSION

The literature shows that the subject of experimental changes in laboratory animals induced by the administration of inorganic compounds of tin, has already been approached in various ways. It covers the different inorganic tin compounds, the quality and quantity of the compounds; routes of administration; species of the experimental animals and the various toxic effects (1, 2, 6, 8, 9, 11, 12). We consider our contribution to be useful however, in view of the fact that we employed a stomach tube for introducing the $SnCl_2$ and a medium for its solution which could, at the same time, be guaranteed as biological harmless.

Deschiens (2), administered $SnCl_2$ i. m. in the therapeutic dose (as anthelmintirum) of 4 mg/kg body weight daily for 8 days and in the lethal dose of 40 to 80 mg/kg body weight daily for 6 days to the guinea pig, rabbit and mouse. Our LD_{50} introduced by means of the stomach tube, was 215 mg/kg body weight (span 158.8–293.8 mg/kg) to white mice. In our experiment we chose this high dose (1000 mg/kg), to ascertain the type of toxic damage and to determine the main changes (clinical, pathological and histological) in the animal organism.

Eight hours after the application of $SnCl_2$ practically all experimental animals were in a premortal state. They were therefore killed to prevent any

post mortem influence on the pathological and histological findings.

Although papers about the toxic action of tin in its inorganic form (compounds) are numerous, reports on the histological and pathological findings are more scarce (3, 4, 14, 15). We have not, as yet, met with any detailed histological findings on animals caused by stannous chloride in the literature available.

The toxic effect of tin administered in the form of stannous chloride by means of a stomach tube, has been established by the pathological and clinical findings in our experiment. The clinical findings were characteristic for an acute intoxication. With $SnCl_2$ the histological findings showed necroses in the liver and in the spleen and thrombi in the liver bed of the vena portae most probably carried through the circulation. No conclusions can be drawn from the findings in the myocardium, as the number of changes were small (only one animal).

SUMMARY

The acute toxic effect of stannous chloride dissolved in pure lemon juice, administered to white mice (average weight 25 g, of standard breed), by means of a stomach tube, in a dose of 1000 mg/kg weight, was observed. Tin caused toxic changes both in the clinical and in the pathological picture.

Z. Pelikán, K. Halačka a E. Černý

AKUTNÍ TOXICKÉ ZMĚNY U BÍLÝCH MYSÍ, VYVOLANÉ APLIKACÍ CHLORIDU CÍNATÉHO

Souhrn

Chlorid cínatý, rozpuštěný ve filtrované citronové šťávě, byl jednorázově aplikován zaludeční sondou bílým myším v dávce 1000 mg/kg váhy. Klinický nález byl charakteristický pro akutní intoxikaci. V pathologicko-anatomickém nálezu a histologickém nálezu šlo především o nekrozy v játrech a ve slezině a o thrombosy v jaterním řečišti vena portae, vyvolané nejpravděpodobněji krevní cestou. Z pokusu vyplývá, že $SnCl_2$ takto aplikovaný, působil u bílých myší zřetelné toxické změny, zejména na játrech a slezině.

З. Пеликан, К. Галачка, Е. Черны

ОСТРЫЕ ТОКСИЧЕСКИЕ ИЗМЕНЕНИЯ У БЕЛЫХ МЫШЕЙ, ВЫЗВАННЫЕ ПРИМЕНЕНИЕМ ХЛОРИСТОГО ОЛОВА

Резюме

Хлористое олово, растворенное в фильтрованном лимонном соке, было отпаторенно через посредством желудочного зонда белым мышам зоной в 1000 мг/кг веса. Данные клинического исследования были характеристическими для острой интоксикации. В патологоанатомической и гистологической картине это была прежде всего некрозы в печени и в селезенке и тромбозы в воротной вене, направление кровотока всего, кровяным путем. Из опыта вытекает, что $SnCl_2$, приготовленный таким образом, вызывает у белых мышей заметные токсические изменения, в особенности в печени и селезенке.

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THE EFFECTS OF METALS ON THE CHICK EMBRYO: TOXICITY AND PRODUCTION OF ABNORMALITIES IN DEVELOPMENT*

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The stimulus for a study of the effects of the various elements on the development of the chick embryo was initiated by the striking effect produced by thallium sulfate injected into the yolk sac of the four-day-old embryo.[1] In order to determine the specificity of this effect, 53 other elements were assayed against the chick embryo. The current status of the work is presented in this report.

Materials and Methods

The effects of the elements were studied principally in the four- and eight-day-old embryo since developmental abnormalities are often grossly evident at these older ages. Furthermore, elements generally toxic to embryos early in development may produce selective growth effects when given to more differentiated embryos. Elements found to produce specific and consistent abnormalities when injected at four or eight days were studied further in younger and older embryos.

Commercially supplied White Leghorn eggs were used. They were maintained at a temperature of 38°C, and candled daily for viability. The volume of the test agent injected per egg ranged from .05 cc. to 0.2 cc. with an occasional yolk sac injection of 0.3 or 0.4 cc. When possible, water soluble salts were used, but, in rare cases, insoluble salts were ground in distilled water and injected as a suspension. The injections were made by two routes: into the yolk, or onto the chorioallantoic membrane (CAM) of eight-day eggs. Yolk sac injections were made through a hole drilled in the blunt end of the egg, and the opening then was sealed with melted paraffin. The CAM was injected through a small window cut in the shell directly over the membrane and the opening was sealed with Scotch tape after the injection.

The preliminary toxicity tests were carried out in the four-day-old embryo. Four eggs were injected at each of three of four dose levels, and, after several trials, the LD_{50} dose was approximated. Groups of six to ten eggs were injected at several dose levels above and below the estimated LD_{50} in order to obtain a more precise figure. In many instances, several confirmatory toxicity runs were necessary to obtain a reasonably consistent LD_{50} figure. With the four-day yolk sac data as a guide, similar toxicity studies were completed in the eight-day embryo by the yolk sac and CAM routes.

The embryos were candled daily and, each day, the dead embryos were recorded and examined. Occasionally, embryos were sacrificed for specific

* This work was supported by grants from the National Cancer Institute of the United States Public Health Service, the Damon Runyon Memorial Fund for Cancer Research and the American Cancer Society.

reasons during incubation but, in most instances, toxicity experiments continued until the 18th day of incubation, when all embryos were sacrificed. At this time, the embryos were weighed and examined grossly for abnormalities of the skin, feathers, bone, brain and internal organs. The membranes and yolk were also inspected.

Results

Toxicity data. The LD_{50} of 54 elements in various forms, when injected into the chick embryo at four and eight days of development, are summarized in TABLE 1. When more than one salt of an element was tested, they are listed with the most toxic salt of that element. There are numerous factors which influence the toxicity of an element in the fertile chicken egg, besides the inherent or direct toxicity of the chemical at its locus of action. These factors are related to:

(*a*) *The egg.* The egg varies in size and chemical composition, the latter being related to the diet of the hen to a considerable extent. Certain excesses or deficiencies of minerals or vitamins, for example, may modify the response of the embryo. An extremely important factor is the developmental age of the embryo at the time the chemical is injected, since the presence of specific organs or metabolic systems may be critical in determining the effect of a chemical.

(*b*) *The state and properties of the injected chemical.* The route of injection and the vehicle used may be very important in the rate of absorption of the chemical. Certain elements may react with non-embryo constituents of the egg, may be precipitated at the site of injection, or may be converted by combining with substances in the egg to form a substance of greater physiological activity.

(*c*) *Distribution and metabolism of the injected chemical.* The rate of absorption and the toxicity of high blood levels of an element are important factors in toxicity. Some chemicals are concentrated in the albumen or in the allantoic sac, and thus detoxified as far as the embryo is concerned. Other chemicals may be deposited in inactive form in some parts of the embryo, as the bones, and thus be rendered less toxic.

All of these factors must be taken into account in interpreting the toxicity or teratogenic effect of an element. In FIGURE 1, the elements have been divided into four groups, based on their toxicity in the four- and eight-day embryo, by the yolk sac and CAM routes. These groupings suggest how some of the factors mentioned above influence the activity of specific chemicals.

Specific Effects on Embryonic Development. Under appropriate circumstances, it is probable that an excess of any element can be shown to have some specific effect on embryonic development. In many instances, however, the element appears to be generally toxic and the embryo either dies promptly or, if the embryo survives, it will develop normally. On the other hand, situations have been found in which some elements will exhibit specific biological activity in the chick embryo. Some elements exert their effect when given in any chemical form, at any time during incubation, and

TABLE 1

TOXICITY OF SALTS OF VARIOUS ELEMENTS IN FOUR- AND EIGHT-DAY CHICK EMBRYOS

Element	Formula of Salt	Estimated LD_{50}			
		Mg/egg	Microatoms/egg*		
		4-day	4-day	8-day	8-day CAM route
		Yolk sac route			
Arsenic	$KAsO_2$	0.02	0.10	0.34	0.07
Arsenic	$Na_2HAsO_4 \cdot 7H_2O$	0.30	0.96	2.60	0.32
Arsenic	As_2O_3	0.16	1.40		
Cadmium	$CdCl_2$	0.02	0.13	0.22	0.09
Selenium	H_2SeO_4	0.03	0.17	0.35	0.11
Platinum	$PtCl_4 \cdot 2HCl \cdot 6H_2O$	0.13	0.25	>19.0	0.17
Lead	$Pb(NO_3)_2$	0.10	0.30	4.50	3.00
Mercury	$HgCl_2$	0.08	0.30	3.10	0.21
Chromium	$Na_2Cr_2O_7 \cdot 2H_2O$	0.06	0.40	0.30	1.02
Chromium	$Cr(NO_3)_3 \cdot 9H_2O$	0.80	2.00	>50.0	7.50
Antimony	$SbCl_3$	0.10	0.44	2.20	0.08
Silver	$AgNO_3$	0.10	0.59	0.50	0.50
Nickel	$NiCl_2 \cdot 6H_2O$	0.20	0.84	10.0	1.40
Vanadium	$VO(CH_3COO)_2$	0.20	1.10	0.65	0.24
Tungsten	Na_2WO_4	0.78	2.60	8.50	6.30
Molybdenum	$Na_2MoO_4 \cdot 2H_2O$	0.50	3.30	2.90	14.0
Copper	$CuCl_2 \cdot 2H_2O$	0.60	3.50	29.0	0.71
Strontium	$Sr(NO_3)_2$	0.80	3.80	>94.0	54.0
Cobalt	$CoCl_2$	0.50	3.80	7.70	2.30
Telluridos	Na_2TeO_3	1.00	4.50	18.0	0.12
Osmium	OsO_4	1.20	4.70	9.90	0.99
Thallium	Tl_2SO_4	1.30	5.20	5.50	2.80
Lanthanum	$LaCl_3 \cdot 7H_2O$	6.00	16.0	54.0	2.80
Zinc	$ZnSO_4 \cdot 7H_2O$	5.00	17.0	>60.0	1.20
Cerium	$Ce(NO_3)_3 \cdot 6H_2O$	8.00	18.0	>46.0	3.00
Manganese	$MnSO_4 \cdot 4H_2O$	5.00	21.0	>86.0	1.50
Uranium	$UO_2(C_2H_3O_2)_2 \cdot 2H_2O$	15.0	22.0	>29.0	0.44
Indium	$InCl_3$	5.00	23.0	>91.0	21.0
Ruthenium	$RuBr_3$	7.00	27.0	>76.0	9.30
Iron	$FeSO_4 \cdot 7H_2O$	8.0	29.0	25.0	2.00
Iron	$FePO_4 \cdot 2H_2O$	15.0	80.0	75.0	2.90
Thorium	$Th(NO_3)_4 \cdot 4H_2O$	20.0	38.0	>38.0	14.0
Fluorine	NaF	1.75	42.0	71.0	30.0
Iridium	$IrCl_3$	14.0	47.0	13.0	1.30
Iodine	KI	8.0	48.0	60.0	78.0
Tantalum	K_2TaF_7	20.0	51.0	36.0	6.40
Tin	$SnCl_2$	10.0	53.0	>105.0	7.40
Tin	$SnCl_4$	20.0	77.0	77.0	5.40
Gallium	$Ga_2(SO_4)_3$	15.0	58.0	77.0	2.70
Magnesium	$MgSO_4 \cdot 7H_2O$	15.0	61.0	>121.0	105.0
Lithium	$LiSO_4 \cdot H_2O$	8.0	62.0	78.0	64.0
Zirconium	$ZrOH(C_2H_3O_2)_3$	18.0	63.0	>70.0	18.0
Boron	H_3BO_3	5.0	81.0	113.0	81.0
Aluminum	$AlCl_3$	15.0	112.0	>150.0	16.0
Silicon	$Na_2Si_2O_5$	20.0	247.0	>247.0	31.0
Germanium	GeI_2	>10.0	>31.0	31.0	3.10
Gold	$AuCl_3HCl \cdot 4H_2O$	>20.0	>41.0	>41.0	1.20
Rhodium	$RhCl_3$	>10.0	>42.0	72.0	4.30
Bismuth	$BiCl_3$	>20.0	>63.0	>63.0	11.0
Yttrium	$Y(NO_3)_3 \cdot 6H_2O$	>30.0	>73.0	>52.0	2.90

TABLE 1 (CONTINUED)

Element	Formula of Salt	Estimated LD_{50}			
		Mg/egg	Microatoms/egg*		
		4-day	4-day	8-day	8-day CAM route
		Yolk sac route			
Barium	$BaCl_2 \cdot 2H_2O$	>20.0	>83.0	45.0	3.30
Palladium	$PdCl_2 \cdot 2H_2O$	>20.0	>94.0	>94.0	19.0
Titanium	$Ti_2(SO_4)_3$	>20.0	>104.0	89.0	20.0
Beryllium	$BeSO_4 \cdot 4H_2O$	>20.0	>113.0	>113.0	5.60
Cesium	$CsCl$	>20.0	>119.0	>119.0	77.0
Rubidium	$RbCl$	>30.0	>248.0	>248.0	124.0
Calcium	$CaCl_2$	>30.0	>270.0	270.0	25.0
Potassium	KCl	>50.0	>671.0	>537.0	134.0
Sodium	$NaCl$	>50.0	>855.0	>685.0	145.0

* Microatoms/egg = $\frac{LD_{50} \text{ in mg/egg} \times 1000}{\text{Mol. wt. of salt in gms.}} \times$ no. atoms of element in salt. The LD_{50} in mg/egg for each salt for the eight-day embryos is calculated easily from the relationship between the mg/egg and microatoms/egg data presented for the four day embryo.

DIAGRAM OF TOXICITY RELATIONSHIPS OF ELEMENTS, AS RELATED TO AGE OF EMBRYO AND ROUTE OF INJECTION

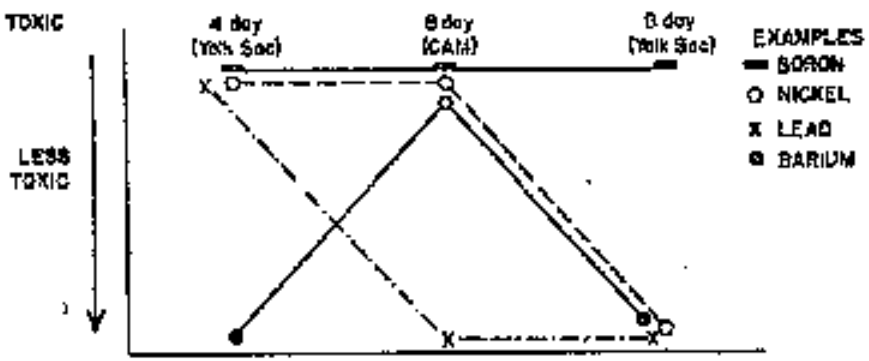

FIGURE 1. Toxicity of elements injected into four- and eight-day-old chick embryos by yolk sac and CAM routes. The four groupings cover the majority of results summarized in TABLE 1. The elements, illustrated by boron, equate approximately the same LD_{50} dose, irrespective of the age or route of administration. This may indicate that the chemical is readily absorbed, and that it acts specifically on a certain system as it appears in the embryo. If this system is not present at the time of injection the chemical remains biologically inactive until the proper susceptible mechanism appears. The elements, illustrated by nickel, show decreased toxicity at eight days as compared to four days by yolk sac injection, but they are toxic on the CAM at eight days. This may be interpreted as indicating that absorption of the salt is more efficient by the CAM route, and as the embryo increases in size more of the salt is necessary to poison it. About 15 per cent of the chemicals tested fall in this category. The elements, illustrated by lead, are most toxic at four days, and less toxic at eight days, by both yolk sac and CAM routes. This suggests that the salt is readily absorbed from the yolk sac as well as the CAM, and the tolerance of the embryo to the chemical increases with its increase in size. The elements, illustrated by barium, are more toxic by the CAM than by the yolk sac route. This pattern was found in about 50 per cent of the elements tested. Chemicals in this group may be precipitated in the yolk sac or they may react with the yolk components so that the embryo is not exposed to the full injected dose. Another possibility is that if the chemical is rapidly absorbed from the CAM, the embryo may be actually intoxicated to a lethal degree, if the mixture is absorbed slowly from the yolk sac, the embryo may detoxify or excrete it before a lethal concentration develops.

by any route of administration, while others are active only when given at specific stages of development, or by a certain route of administration, or in a definite chemical form. During the course of our toxicity studies, if gross and definite evidence of embryonic abnormality was produced by a given element, the effect was analyzed in more detail. If nothing specific occurred, possibly because an abnormality was missed by our gross methods of examination, or because the embryo was not treated properly to bring out such an effect, further studies have not been carried out. It does not mean, of course, that the element is completely without teratogenic activity. Nine elements thus far have been found to produce a specific and reproducible effect on embryonic development, and these are summarized in TABLE 2.

TABLE 2

SUMMARY OF ELEMENTS PRODUCING ABNORMALITIES IN EMBRYONIC DEVELOPMENT

Element	*Abnormality*	*Maximum activity*		*Approximate consistency of effect, per cent treated embryo*
		Age of embryo at injection	*Route of injection*	
Thallium	achondroplasia	4–10	yolk sac, CAM	90–100
Chromium	mild achondroplasia	8	CAM	60
Lead	brain injury	3–15	yolk sac, CAM	80–90
Cobalt	brain injury	4–12	yolk sac	80–90
Boron	pale and edematous, feather inhibition	0–5	yolk sac, CAM	70–85
Arsenic	stunted, micromelia, abdominal edema	4	yolk sac	50
Rhodium	stunted, feather inhibition, micromelia, mild edema	8	CAM	30
Barium	abnormal feet	8	yolk sac, CAM	50
Selenium	bizarre abnormalities, stunting	0–8	yolk sac, CAM	10–30

(*a*) *Thallium.* Thallium sulfate produced a severe and consistent picture of achondroplasia in the chick embryo,[3] which was evident in embryos sacrificed or dying at ten days of incubation or later. The most marked effects were seen when thallium was given into the yolk sac at four to ten days of incubation, and the LD_{50} dose during this period was between one and two mg/egg. Thallium did not produce achondroplasia when given at the maximum tolerated dose (0.3 mg/egg) to the two day embryo, presumably because thallium had a generally toxic effect at higher doses at two days, and lower doses were insufficient to produce achondroplasia. The LD_{50} of thallium sulfate on the CAM at eight days is 0.7 mg/egg, and achondroplasia occurs consistently at doses of 0.4 to 0.7 mg/egg by this route. Thallium produces a less severe achondroplasia in embryos injected after the tenth day.

When sacrificed at 18 to 20 days, the embryo treated with thallium at four to eight days is severely achondroplastic, the limbs are short and the

long bones curved, the trunk is thickened, the head is small and the beak resembles a parrot's. On gross examination of the head, the eyes and orbit appear smaller than normal. The brain is small, sometimes contains cysts and the cerebellum may be atrophied. The abnormalities in the central nervous system are presumably due to compression of the brain and eyes as a result of defective growth of the skull. The feathers appear normal (FIGURE 2A). Roentgenograms of the thallium-treated embryos show the conspicuous interference in bone growth (FIGURE 2D, E). Microscopic section of the toe of the thallium-treated embryos shows defective cartilage growth, with failure of longitudinal bone growth, but the shaft is widened and thickened by the deposition of bone in the shaft (FIGURE 2B, C).

(*b*) *Chromium.* Sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) produced no apparent abnormalities when injected into the yolk sac of the four-day embryo. When sodium dichromate was injected onto the CAM, however, about 60 per cent of the embryos developed moderate achondroplasia, parrot beaks, slight stunting in growth, paleness and, in some instances, sparse feather formation (FIGURE 7C). Achondroplasia was never as severe as that produced by thallium. Injection of sodium dichromate into the yolk sac at eight days, and chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) into the four- and eight-day embryo failed to produce gross developmental abnormalities.

(*c*) *Lead.* The effect of lead on the early development of the chick embryo has been the subject of several studies, including those of Hammett and Wallace[2] and Catizone and Gray[4]; the latter group, working in the 0 to 48 hour of incubation period, concluded that lead can specifically affect the morphogenesis of the central nervous system of the chick. Our studies were carried out in older embryos, and lead was found to have a remarkable effect on the central nervous system. Four-day-old embryos injected with 0.15 mg. of lead nitrate showed an extreme degree of hydrocephalus when examined at 18 days of age[4]. In most instances, following the yolk sac injection of 0.15 mg/egg of lead nitrate at four days, embryos dying or sacrificed within the next four days exhibited brain hemorrhage (FIGURES 3A, B). Embryos examined after eight days of incubation presented varying degrees of hydrocephalus, ranging from a small herniation of the meninges to massive enlargement of the head (FIGURE 4A, B). After the tenth day of incubation, some of the brains showed evidence of regeneration of nervous tissue, particularly at the base and anterior portion.

Lead produced brain hemorrhage when injected into the yolk sac over the four- to 15-day incubation period. At eight days, doses of 0.1 mg/egg or higher of lead nitrate produced brain injury in 96 per cent of the embryos. These embryos usually showed necrosis of the brain substance at 18 days, and only a thin shell of nervous tissue remained, distended by a bloody fluid. Although the tolerance of the embryo for lead increased rapidly, the dose injuring the brain was fairly constant during the fourth to eighth day. At the tenth day and beyond, larger doses of lead nitrate, up to two mg/egg, were necessary to induce brain injury. This was attributed to the fact that other systems, such as bone, were appearing in the chick embryo, and the lead may have been competitively fixed by these new systems. The

FIGURE 2. Effect of thallium on the chick embryo. A—Embryo, sacrificed at 18 days, injected with one mg. of thallium sulfate into the yolk sac at four days. B—Embryo shows characteristic picture of anencephalus, with shortened extremities and parrot beak. B—Microscopic section of toe of 18-day-old embryo. C—Toe of 18-day embryo treated at four days with thallium. The thallium-treated toe is shortened, curved and bone formation impaired, but there is some bone formation in the shaft. D—Roentgenogram of a normal 18-day embryo. E—An 18-day thallium-treated embryo. The bones of the extremities are shortened, curved and appear denser than the control.

histopathology of the brain injury induced by lead is now under study,[4] but the sequence of events, as they are seen grossly, are extensive hemorrhage throughout the brain substance within 48 hours after injection, followed by

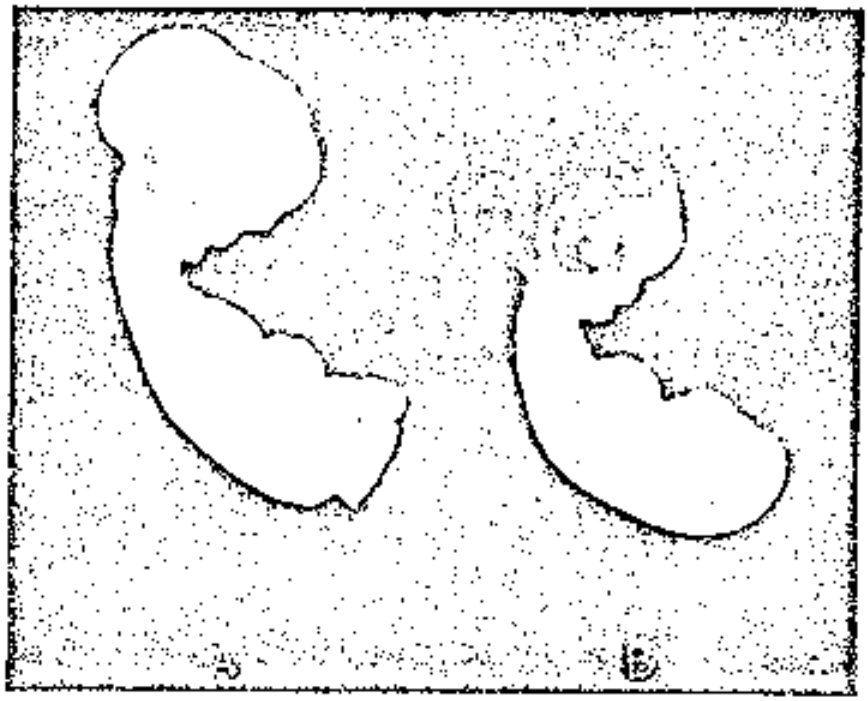

FIGURE 3. A—Control. B—Lead-treated embryo six days of age. Injected in the yolk sac with 0.13 mg. lead nitrate at four days of age. Marked hemorrhage is present in the brain.

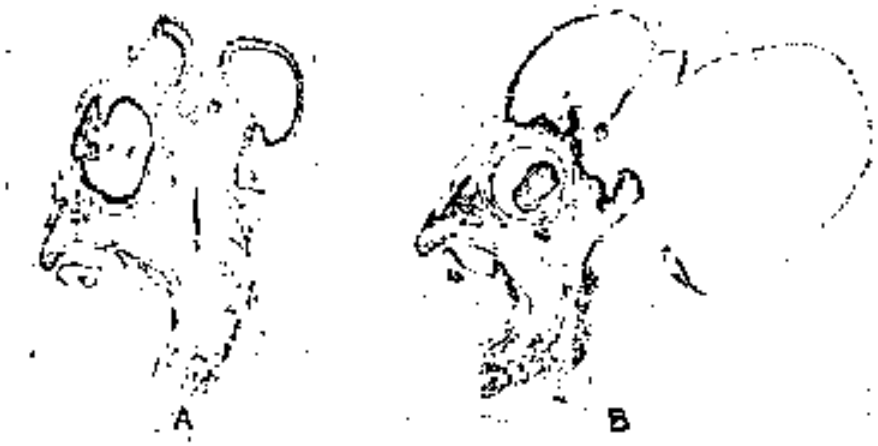

FIGURE 4. Microscopic sections of head of control A, and lead-treated embryo B, eight days of age. B was injected in the yolk sac with 0.13 mg. lead nitrate at four days. The cranium of the lead-treated embryo is enlarged and empty, as a result of the hemorrhagic necrosis in the brain induced by lead.

necrosis of the tissue. Some recovery is seen later, particularly if brain injury was induced early in development.

Lead nitrate failed to induce gross evidence of brain injury when injected into the yolk sac at two days of incubation, whereas in the three-day embryo, brain injury occurred in 70 per cent, and, in the four-day embryo, in 85 to 90 per cent of the treated embryos. It is of interest that the period of development when lead begins to induce its effects on the brain coincided with the appearance of blood vessels in the nervous system.[6]

The effects of lead and thallium could be induced by the simultaneous

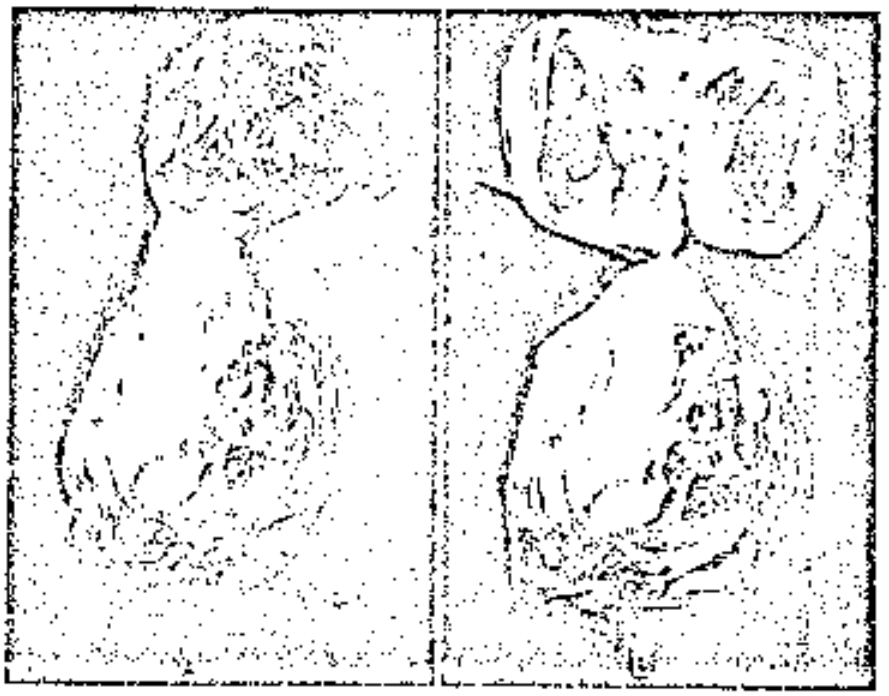

FIGURE 5. Embryo, 18 days of age, injected with one mg. of thallium sulfate into the yolk sac at four days, and 0.33 mg. of lead nitrate into the yolk sac at eight days of age. Note the acrania-dysraphia and hydrocephalus (A), and the distended empty cranium (B). A similar picture followed the simultaneous injection of both agents at eight days of age.

injection of lead nitrate and thallium sulfate into the same embryo (FIGURES 5A, B).

(*d*) *Cobalt.* Gray and Scully[4] reported that cobalt chloride, injected into the albumen of the egg before incubation, did not induce any specific abnormalities in the embryo, but it appeared to inhibit the growth of the amnion. In our work, cobalt has been used later in development. Cobalt chloride injected into the yolk sac of eight-day embryos consistently induced softening and some hemorrhage in the brain, when it was examined at 16 to 18 days (FIGURE 6A, B). The embryos also were pale, slightly edematous and the livers were enlarged. The nature of the brain injury is under study. Cobalt induced brain injury, apparently less severe than at eight days, when given at four or 12 days of development.

(*e*) *Boron.* When given in the yolk sac of the four-day-old embryo as boric acid, boron caused marked growth inhibition, and embryos surviving

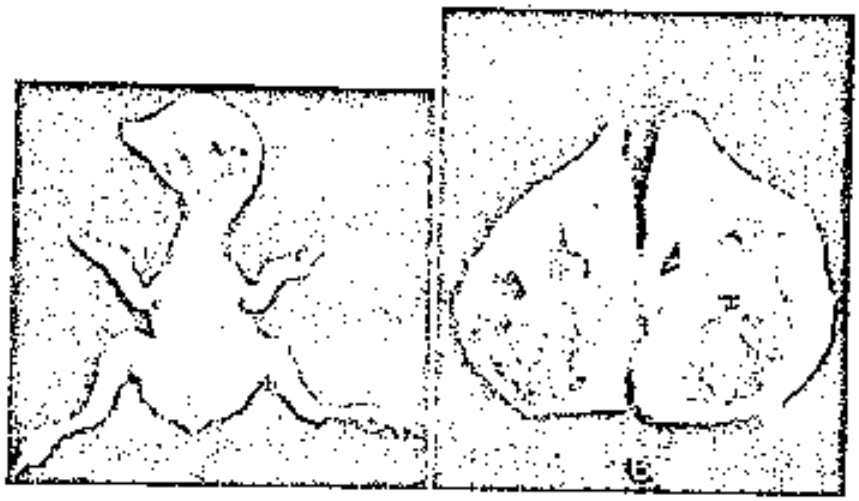

FIGURE 6. A. 18-day-embryo, after injection in the yolk sac at eight days with two mg. cobalt chloride. Embryo shows redness and pallness of the head, impaired feather growth, mild generalized edema and slight micromelia. B—Splitting the head reveals a soft brain with some hemorrhagic areas.

FIGURE 7. A—Embryo sacrificed at 18 days, injected on the CAM at eight days with five mg. boric acid. The embryo is pale and stunted, and shows an overall gelatinous edema and scanty — if initiated feather growth. B—Embryo, sacrificed at 18 days of age, was injected with two mg. of mercury chloride onto the CAM at eight days. The embryo is severely stunted with mild micromelia and impaired feather formation. C—Embryo, sacrificed at 18 days, was injected onto the CAM with 0.1 mg. of sodium dichromate (Na$_2$CrO$_4$·2H$_2$O) at eight days of age. The embryo shows a moderate acrocephalosyndactyly with parrot beak, and slight growth inhibition.

to 18 days were also very pale, showed definite interference in feather growth and occasional beak deformities. Boric acid, by the yolk sac and CAM routes at eight days, caused a similar effect on the embryos, and those

surviving to 18 days also showed an over-all gelatinous edema (FIGURE 7A). The LD_{50} dose of boric acid given into the yolk sac at zero and two days of age was in the same range as the four- and eight-day dosage, five mg/egg, and the effects on the embryo when examined at 18 days were somewhat similar, irrespective of the age of the embryo when injected. Boron, therefore, caused a specific and consistent effect on the chick embryo when given at zero to eight days at a fairly uniform dose level.

Arsenic. Sodium ortho arsenate ($Na_2HAsO_4 \cdot 7H_2O$), a pentavalent arsenical, produced definite growth inhibition when injected into the yolk sac

FIGURE 8. A—Embryo, sacrificed at 18 days, injected into the yolk sac at four days of age with 0.20 mg. of an arsenical salt ($Na_2HAsO_4 \cdot 7H_2O$). The embryo is markedly stunted, shows mild micromelia, impaired feather growth and an edema of the abdomen, as compared with B, a normal 18-day-old chick embryo.

of four-day embryos, but not in the eight-day embryo. At 18 days, the embryos were small, showed mild micromelia and abdominal edema (FIGURE 8A). Potassium arsenite ($KAsO_2$) and arsenic pentoxide (As_2O_5) did not produce this picture when given at four or eight days.

Rhodium. Rhodium chloride produced abnormal embryos when injected on the CAM at eight days. The embryos surviving to 18 days were somewhat similar to those treated with sodium ortho arsenate, in that they were stunted, with mild micromelia and edema, but the rhodium treated embryos showed greater interference with feather growth (FIGURE 7B).

Barium. Barium chloride, injected into the yolk sac or onto the CAM of the eight-day embryo, produced a remarkable inhibition in the growth of the toes (FIGURE 9A). This abnormality occurred irregularly, but was seen in about 50 per cent of the treated embryos surviving to 18 days.

FIGURE 9. Feet of 18 day embryos. A—After injection in the yolk sac at eight days with 20 mg. barium chloride, and showing deformity and inhibition in toe development as compared with B—control normal toes.

FIGURE 10. Effect of selenium on the chick embryo. A—18-day embryo injected in the yolk sac before incubation with 0.01 mg. selenious acid (Effecth). This embryo shows malformation of beak and upper beak. B—18-day embryo injected in the yolk sac at eight days with 0.05 mg. of selenious acid. The embryo developed apparently normally, except for the enormous cysts on the pump.

There were no other gross effects noted on the embryo. None of the embryos injected at 4 days showed toe abnormalities.

Selenium. Franke, *et al.*[8] obtained a variety of monsters following the injection of sodium selenate into the air sac prior to incubation. We obtained several abnormal embryos, two of which are shown in FIGURE 10A, B, when selenious acid (H_2SeO_3) was injected into the yolk sac of embryos from zero to eight days of age. The incidence of abnormalities in our experience was low, in the range of 10 to 20 per cent of the embryos surviving to 18 days.

Conclusion

The effects of excessive concentrations of elements on the developing chick embryo may be a useful method for studying the biological properties of the elements. It is apparent that some elements, at least, induce a characteristic effect on the embryo when given at the proper period of development, and two elements, given simultaneously, may each induce their specific abnormality in the embryo. Some of the elements have caused destruction and regression in an organ already well-developed at the time of treatment, thus demonstrating a teratogenic effect by retrogression of an established organ, rather than by damage to the anlage of a developing organ. The specific affinity of some elements and embryonic organs, as suggested by selective injury to these organs, may suggest approaches and methods for damaging neoplastic growths arising from those organs.

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Effects of Oral Administration of Two Tin Compounds to Rats over Prolonged Periods

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Abstract—Three malignant tumours were seen in 30 August rats (13 male and 17 female) which survived for 1 yr or more on a diet containing 2% sodium chlorostannate. One tumour was an adenocarcinoma of mammary origin, another a pleomorphic sarcoma arising in the uterus and the third, an adenocarcinoma arising in the region of the jaw. In 29 rats (13 males and 16 females) which survived for 1 yr or more on a diet containing at first 1% then 0·5% stannous 2-ethyl hexoate, no neoplasms were seen. In 33 rats (16 males and 17 females) fed on a control diet for the same period no malignant tumours were seen. Chronic murine pneumonia with complicating bronchiectasis was present in most rats at death. The difference in tumour incidence between the 3 groups is regarded as being probably without significance, but a further larger experiment would be necessary to establish this with certainty.

INTRODUCTION

The reasons for undertaking this experimental work have been given and the background of previous studies against which it was undertaken in the previous paper (Walters & Roe, 1965).

EXPERIMENTAL

Materials. Sodium chlorostannate was obtained from the British Drug Houses Ltd., Poole, Dorset and stannous 2-ethyl hexoate from Theodore St. Just & Co., Whitfield, Manchester.

Animals. Inbred August hooded rats of both sexes were used. Mothers were fed the diets from the day their litters were born. The young were weaned at 4 weeks and transferred to fresh cages with 8–10 males or females per cage. Cages made of zinc were used, both for breeding and for subsequent housing. Water was provided *ad lib.* throughout the experiment.

Basic diet. A powdered diet was supplied by Messrs Dixon, Ware, Herts. It was supplemented by the addition of arachis oil (5%) and certain vitamins and minerals. The formula of the resulting 20% protein basic diet is given by Walters & Roe (1965).

Test diets. Sodium chlorostannate was dissolved in water and the solution thoroughly mixed with the powdered diet in an electric mixer for a period of 10 min to make a dough. Stannous 2-ethyl hexoate was added to the diet essentially in a similar manner, except that it was dissolved in arachis oil instead of water before being mixed in. A rounded mass of the dough was placed in each cage of rats, the mass being in excess of their daily requirement. Diets were made up in dough form twice weekly but fed daily during the week. A double quantity was provided on Saturdays, but none on Sundays.

Conduct of experiment. Animals were examined cursorily each day except Sundays, and weighed and thoroughly examined each week. Sick animals were killed and examined *post-mortem* for tumour development and other changes.

Experimental design. Pregnant August rats were divided at random into 3 treatment groups. From the day of birth of litters onwards, groups 1 and 2 were fed for up to 80 weeks on a 20% protein diet containing 2% sodium chlorostannate and 1% stannous 2-ethyl hexoate, respectively; group 3 rats were fed on an untreated 20% protein diet for the same period. According to the initial plan of the experiment, the young raised by mothers fed on the 3 different diets were, at the time of weaning, to be separated by sex and thereafter fed the same diets continuously until death. The numbers of animals in the 3 groups at weaning were: group 1—37 (19 males and 18 females), group 2—37 (17 males and 20 females), group 3—40 (20 males and 20 females).

RESULTS

Unfortunately, the plan of the experiment could not be followed absolutely. Rats of group 2 were severely underweight and macroscopically obviously anaemic at the time of weaning. However, the diet containing 1% stannous 2-ethyl hexoate was still fed to them. By week 8 the average body weight of males of group 2 was 64 g and of females, 74 g. At this time control males in group 3 averaged 146 g and control females 116 g. Rats in group 1, on the other hand, showed no ill effects and were almost as sturdy as the controls, the males averaging 135 g and the females, 105 g.

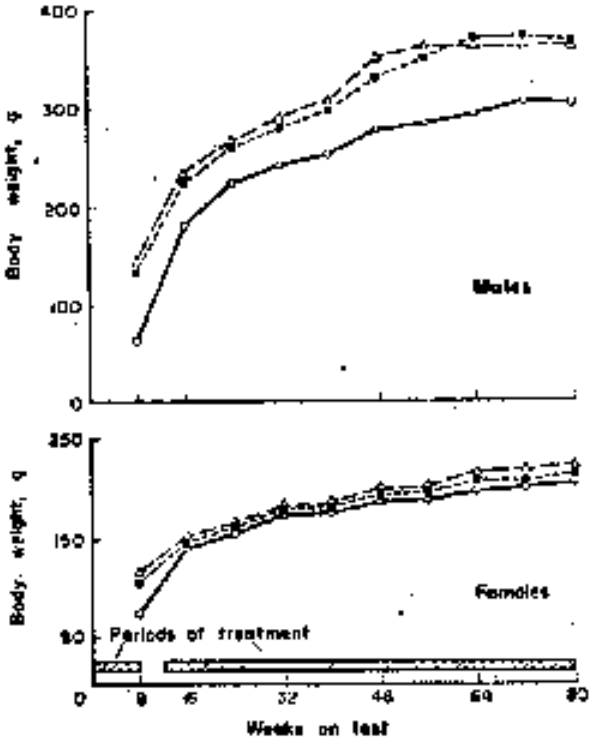

FIG. 1. Body weight gains in males and females of rats receiving 20% protein diets containing 2% sodium chlorostannate (●——●), 1% stannous 2-ethyl hexoate (O——O); or no tin compound, control, (△——△).

Because of the poor condition of rats in group 2, they were given the control diet for 4 weeks after which their condition had greatly improved and their body weights were not much less than the controls. They were then returned to a diet containing 0·5%, instead of 1·0% stannous 2-ethyl-hexoate and remained on this diet until death.

Although there was no evidence of toxic effect, rats of group 1 were also taken off treatment with the test substance from weeks 8–12. However, on resumption of treatment with sodium chlorostannate the level of incorporation into the diet remained, as before, at 2%.

The periods of treatment and the pattern of increase in weight gain in the 3 groups are illustrated in Fig. 1. During the first year of the experiment there were 24 deaths as follows: 7 in group 1 (6 males and 1 female), 10 in group 2 (6 males and 4 females), and 7 in group 3 (4 males and 3 females). Evidence of chronic murine pneumonia was present in most of these animals and was the primary cause of death or reason for being killed, in most cases. No other pathological change or neoplastic lesion was observed.

Chronic respiratory disease was evident in the majority of rats of all groups alive at 1 yr and remained in evidence until the survivors were finally killed at 80 weeks.

The results of the experiment as regards survival beyond week 50, and neoplastic lesions in animals so surviving, are summarized in Table 1. The majority of rats in all 3 groups showed evidence of moderate or severe lung infection with wide cuffs of mononuclear cells around bronchi and vessels, and, in many cases, bronchiectatic changes. Slight degenerative changes were observed microscopically in some of the livers of animals of all 3 groups and were not more frequent in the groups receiving tin. No hyperplastic nor neoplastic lesions were seen in the gastro-intestinal tract. Areas of dilated tubules were seen in the renal cortices of a few rats of each of the 3 groups. There was no other significant pathological finding.

Table 1. *Treatment, survival, and post-mortem findings*

Group	Treatment	No. of survivors at week 8*	52	80	Neoplasms found at necropsy in animals dying during weeks 52–80
1	2% Sodium chlorostannate (5000 ppm Sn) in 20% protein diet from birth till week 8 and from week 12 until death	19M	13M	8M	Adenocarcinoma arising in region of jaw
		18F	17F	16F	Pleomorphic sarcoma of uterus Mammary adenocarcinoma
2	1% Stannous 2-ethyl hexoate (4500 ppm Sn) in 20% protein diet from birth till week 8 then 0·5% (2250 ppm Sn) until death	17M	11M	6M	None
		20F	16F	14F	None
3 (Control)	20% Protein diet only	20M	16M	12M	None
		20F	17F	16F	Lymphosarcoma of lung

M = Male F = Female

* There were no deaths up till week 8.

The 3 malignant tumours seen in group 1 are illustrated in Figs. 2–4.

DISCUSSION

The result of the experiment is, unfortunately, inconclusive. It is possible, perhaps probable, that all 3 malignant tumours in rats of group 1 were spontaneous in origin, and that the high intake of tin played no causative role. This problem could not be settled without the use of much larger groups of animals, and our knowledge and past experience of the August hooded strain used is insufficient to be helpful.

The lack of any apparent changes in the gastro-intestinal tract, and the lack of an observable difference between the livers and kidneys of treated and control groups is encouraging, since these are the sites most likely to be affected by a dietary carcinogen. The lack of any evidence of carcinogenicity of tin in mice (Walters & Roe, 1965) is similarly encouraging.

Although pulmonary disease was rife in all 3 groups and reduced survival to 80 weeks there is no reason to think that the presence of this disease interferes with the response of animals to carcinogenic agents to the extent of changing a positive result to a negative one, or *vice versa*.

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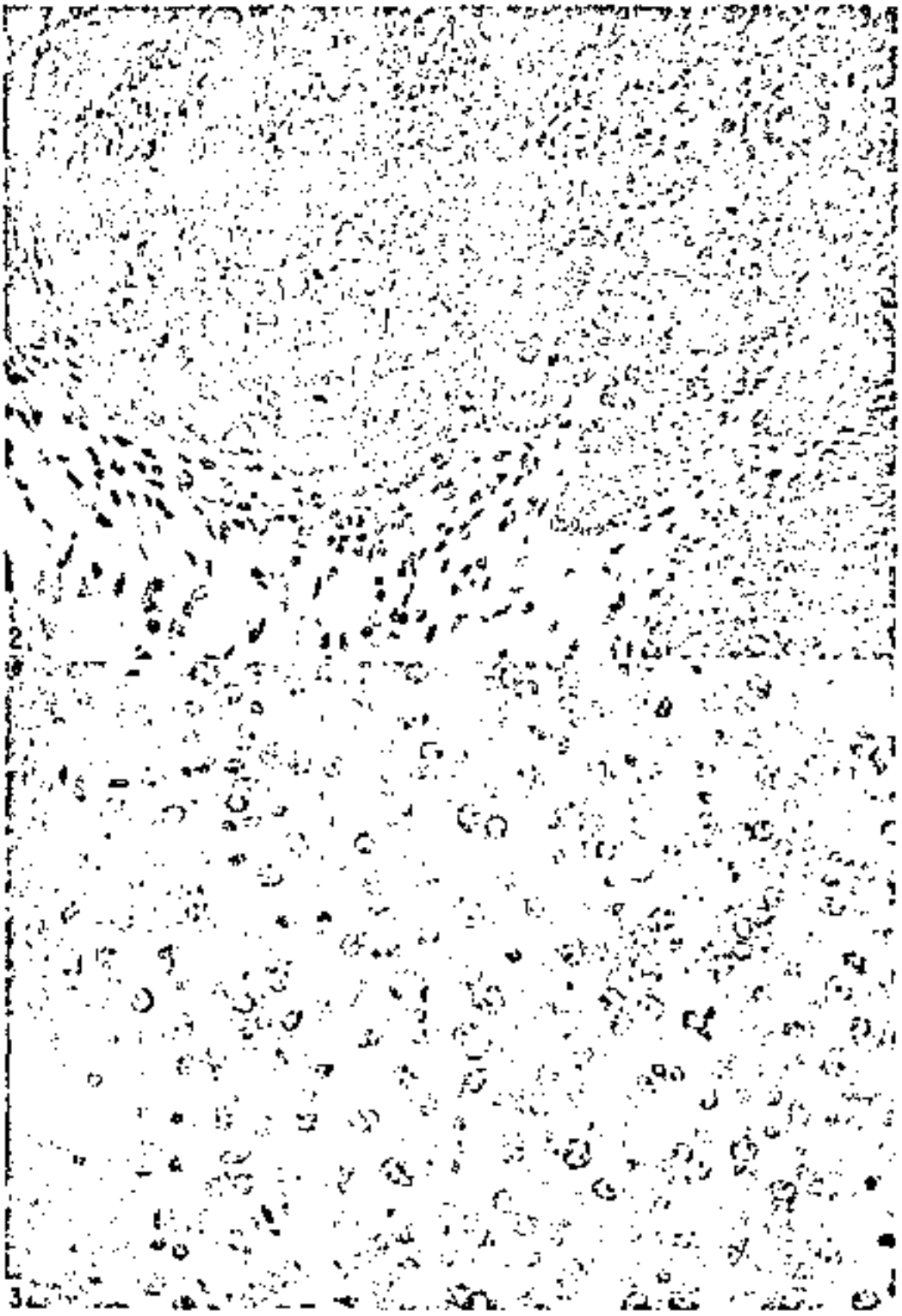
Effets de l'Administration Orale de Deux Composés de l'Étain à des Rats Pendant des Périodes Prolongées

Résumé—Trois tumeurs malignes apparurent chez 30 rats nés au mois d'août (13 mâles et 17 femelles) qui supportèrent pendant un an ou plus un régime contenant 2% de chlorostannate de sodium. L'une des tumeurs était un adénocarcinome d'origine mammaire, une autre, un sarcome pléomorphique de l'utérus, et la troisième, un adénocarcinome de la région de la mâchoire. Sur 27 rats (11 mâles et 16 femelles) qui survécurent un an ou plus avec un régime contenant d'abord 1% puis 0,5% de 2-éthyl hexoate stanneux, on ne constata aucun néoplasme. Sur 33 rats (16 mâles et 17 femelles) gardés sous contrôle alimentaire pendant la même période, on ne détecta aucune tumeur maligne. Il existait de la pneumonie murine chronique compliquée de bronchiectasie chez la plupart des rats, à la mort. La différence dans le nombre des tumeurs entre les trois groupes est considérée comme sans signification probable, mais on ne pourrait conclure avec certitude qu'après des expériences à plus grande échelle.

Wirkung Zweier Zinnverbindungen bei Ratten bei Längerer Oraler Verabreichung

Zusammenfassung—An 30 Augustratten (13 männlichen und 17 weiblichen), die ein Jahr oder länger die Verabreichung eines Futters mit einem Gehalt von 2% Natriumchlorostannat überlebten, wurden drei maligne Tumoren festgestellt. Ein Tumor war ein Adenocarcinom mammaren Ursprungs der zweite ein pleomorphes Sarkom im Uterus und der dritte ein Adenocarcinom im Kieferbereich. Bei 27 Ratten (11 männlichen und 16 weiblichen), die ein Jahr oder länger die Verabreichung eines Futters mit einem Gehalt von zuerst 1% dann 0,5% Stanno-2-äthylhexoat überlebten, wurden keine Neoplasmen gefunden. Bei 33 Ratten (16 männlichen und 17 weiblichen), die ebenso lange eine Kontrolldiät erhielten, wurden keine malignen Tumoren festgestellt. Beim Tode wurde bei den meisten Tieren Rattenpneumonie mit Bronchiektasie als Komplikation gefunden. Der Unterschied in der Tumorhäufigkeit bei den drei Gruppen ist wahrscheinlich nicht signifikant, doch wäre eine weitere ausgedehntere Untersuchung erforderlich, um dies mit Sicherheit feststellen zu können.



◀

FIG. 2. Adenocarcinoma from jaw region of a male rat of group I (2% sodium chlorostannate) killed during week 80 of the experiment. The tumour was thought to have arisen in one of the adnexal glands of the skin. Haematoxylin and eosin × 400.

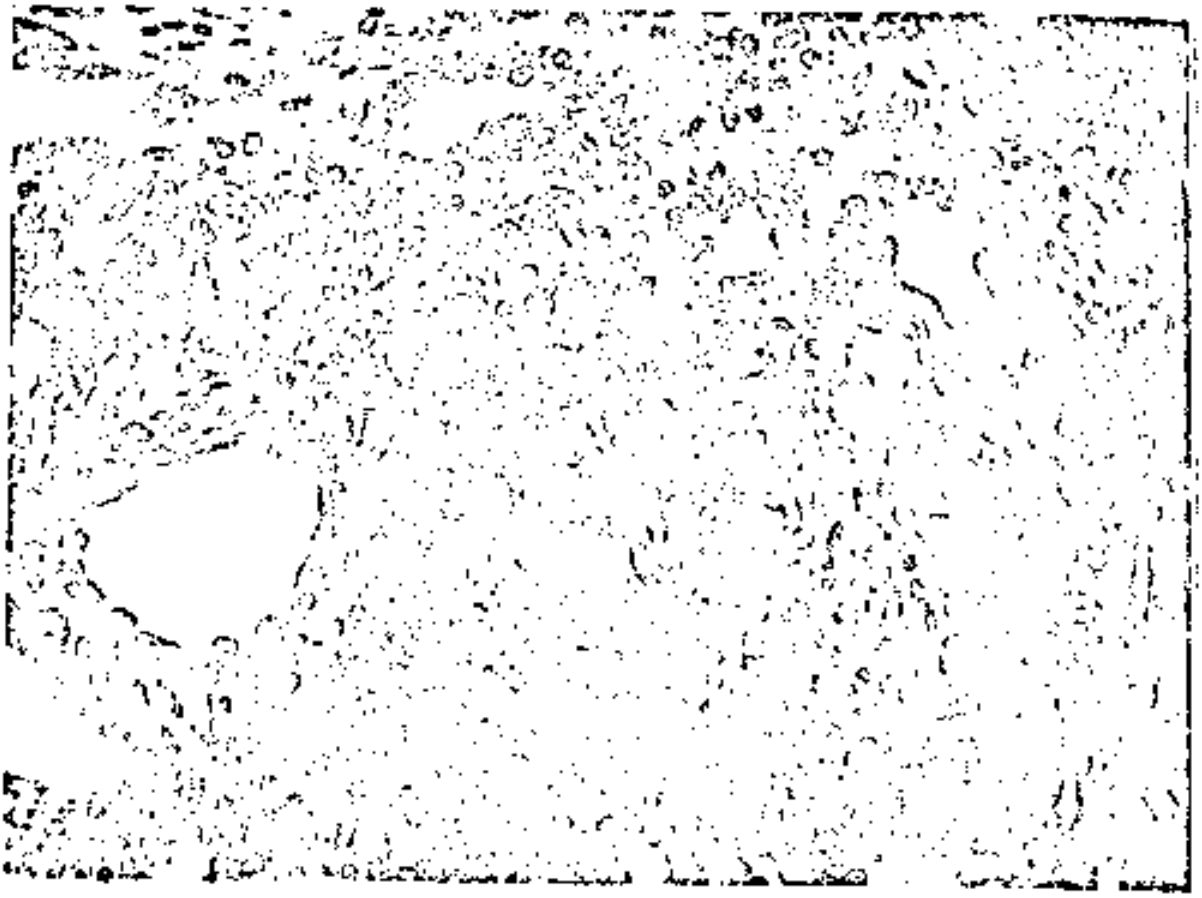


FIG. 4. Adenocarcinoma of mammary gland origin found in a rat of group I (2% sodium chlorostannate) killed during week 80 of the experiment. Haematoxylin and eosin × 400.

◀

FIG. 3. Pleomorphic sarcoma of the uterus in a rat of group I (2% sodium chlorostannate) killed during week 80 of the experiment. The cells showed marked variation in size, abundant pale eosinophilic cytoplasm and frequent, often abnormal, mitotic figures. Haematoxylin and eosin × 400.

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ABNORMAL TRACE METALS IN MAN: TIN*

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As a biologically interesting trace metal, tin has been largely neglected since the turn of the century. Ingested tin in usual amounts was assumed to be nontoxic. Human exposures were believed to come mainly from tinned food. Overt toxicity rarely has been described. Notable biological effects on living systems or their components are generally unknown.

And yet from a theoretical standpoint, tin is one non-ubiquitous metal to which civilized man has long been exposed and which accumulates in his tissues in widely varying amounts. The bodily content of the average American adult exceeds those of the essential or probably essential metals, manganese, chromium, molybdenum, vanadium and cobalt, and those of the apparently non-essential ones, cadmium, barium, titanium, nickel and zirconium. If one were to suspect trace metals of playing etiological roles in degenerative diseases of civilized man, tin would be among the first to be surveyed.

Tin has played an important role in civilization since the beginning of the Bronze Age. The unknown metallurgist who first mixed tin with copper in proportions of 1 : 9 made a discovery which changed the course of history. For the first time a workable hard metal with a low-melting point was available to fabricate durable weapons, ornaments, coins, cooking utensils, bells and statuary.‡ Mining and smelting became established industries, adjacent cities grew wealthy, the science of navigation advanced, trade flourished, trading centers expanded, a capitalistic economy developed, new lands were discovered and much of Europe and the Near East was opened to commerce. The wealth of the city of Tarshish probably was founded on Spanish tin and copper. As mines were depleted, exploration for new sources stimulated voyages of discovery and trade with Britain for Cornish tin (still being mined) and with Central Europe for Moravian tin became essential. This thousand-year-old multifaceted civilization declined with the advent of ironmongering.

The second great tin revolution began in 1810, when tin plate was first used for

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‡Michelangelo, in speaking of sculpture, said that clay represents life, plaster death and bronze immortality. The survival of artifacts for 3500 years testifies to the material aspect of the immortality of bronze.

canning foods. Responding to the military needs of the Napoleonic Wars, Peter Durand, an Englishman, developed the discovery of Nicholas Appert, a Frenchman, that foods could be preserved by boiling and corking the containers [1]. This invention eventually changed the nutrition of much of the human race, although five decades were to elapse before canning of food became prevalent. Concurrently an enormous increase in human exposures to tin spread first throughout North America and later throughout the civilized world. Although the present domestic consumption of tin is about the same as it was in 1910 [2], enough tin plate for canning is used yearly to make a 100 foot-wide belt around the earth at the equator. Because most of the major industrial nations mine little or no tin, political and economic pressures have been exerted on major tin-producing areas of the world (Asia supplies 65 per cent, South America and Africa 11 per cent each, of the world's tin). Widespread human exposures to tinned food, therefore, have lasted about 100 years and have become relatively universal for about 50 years.

The purpose of this communication, the seventh of a series [3–8], is to examine sources and exposures of man to tin as objectively as possible. Six questions were asked: (1) Is tin an invariable component of human tissues? (2) To what extent does tin accumulate with age? (3) Does tin occur in wild animals? (4) What fresh foods contain tin? (5) What is the evidence that tin is nontoxic? (6) Does tin behave in the biosphere as an essential trace metal or as an industrial contaminant?

METHODS

Samples were ashed at 450°C in a muffle furnace after drying at 110°C to constant weight. Tin was first measured by the method of SANDELL [9], using toluene 3,4-dithiol. When a new batch of dithiol was synthesized by the manufacturer it was found unsatisfactory, producing heavy colloidal cloudiness in the sample whereby optical density was impossible to measure. Therefore, most of the analyses were made by the phenylfluorone method of THOMPSON and MCCLELLAN [10]. Comparison of 5 analyses by the two methods gave almost identical results. Accuracy of the latter method was better than 98 per cent, recoveries of known amounts added to blanks or unknowns averaged 100 per cent and as little as 5.0 μg could be detected, or 0.5–0.05 μg/g wet weight. In the tables, 0.0 means less than 0.05 μg/g.

Analytical results on human tissues from around the world were obtained by spectrographic methods previously published [11]. The raw data of TIPTON and COOK on American tissues [12] and of TIPTON *et al.* on foreign tissues [13] were surveyed individually by age and area. Recoveries of known amounts varied from 75 to 92.5 per cent, depending upon the concentration, and the method was sensitive to less than 5 p.p.m. ash. In the tables, 0 means less than 5 p.p.m. ash.

Most samples of food were obtained from three sources: (1) A home garden never fertilized supplied some vegetables. Rye was grown on fallow ground on poor land cleared of trees in a remote forest. Samples of vegetation came from the forest. (2) The truck garden of this institution supplied other vegetables. (3) Other foods were purchased locally from national chain stores. Wild animals were obtained by shooting or trapping.

RESULTS

Organ specificity of tin in human tissues

Tin was found in every one of 29 organs and tissues of American adults. The highest

concentrations were in the intestinal tract where 92 per cent of 637 samples showed it. Median concentrations altered caudally as follows (p.p.m. ash): esophagus 18 stomach 16, duodenum 28, jejunum 36, ileum 79, cecum 130, sigmoid colon 45, rectum 57. This interesting pattern appears related to reabsorption of fluids from intestinal contents.

Sizeable amounts of tin were found in lung, adrenal and liver, with 37, 23 and 23 p.p.m. respectively. Tin was seldom detected in brain (5.4 per cent) or muscle (19 per cent), while diaphragm, heart and pancreas had low concentrations. In no single tissue, however, was tin always found, although all subjects showed it in many organs. Bone accumulated variable to large amounts.

Tin, therefore, was widely but not universally distributed through almost all American human tissues, with a predilection for intestine and lung.

Paired rank correlations of concentrations of organ tin with those of other metals were made by digital computer. Few highly significant ($r>0.50$; $P<0.001$) correlation coefficients were found for any tissue. Only silver in skin, iron in larynx, barium, calcium and manganese in rectum and nickel in cecum appeared to accumulate with tin to a significant degree. Concentrations of tin and lead were associated only in esophagus ($r=0.37$; $P<0.001$). In the aorta, which often contains few abnormal trace metals [12], tin ranked fifth in prevalence (after aluminum, strontium, lead and barium) and fourth in abundance, being found in 95 per cent of 130 specimens at a median concentration of 14 p.p.m. ash.

Individual variations in tissue tin

In viewing the raw data, we were impressed by the occasional adult cases with very high or very low concentrations. For example, in aorta, kidney and liver there were 3 samples with levels greater than 150 p.p.m. ash (1.65 p.p.m. wet weight), and in lung 9 samples, 2 of them with more than 880 p.p.m. Five samples from the gastrointestinal tract contained more than 1000 p.p.m. On the other hand, in 100–145 samples, there were 5 adult lungs, 7 livers, 9 aortas, 23 spleens, 66 pancreases and 17 kidneys with undetectable to trace amounts. Even 20 of 438 samples of intestinal tract were devoid of tin, as were 30 of 199 samples of esophagus and stomach. Therefore exposures probably can vary considerably from one individual to another.

Variations in tissue tin according to age

In Table I are shown tin concentrations in kidney, liver, lung and ileum by decade of life. Values for infants and children are divided into three groups: stillborn, infants up to one year of age and children up to 10 years. Little or none was found in the stillborn, only one lung showing it (with a trace in two intestines), while some infants appeared to be exposed relatively early in life (within days). Children sometimes had higher tissue levels than young adults, 4 kidneys and 4 livers exhibiting more than 150 p.p.m. ash. Because of the wide individual variations, ranges are not shown. Therefore, tin does not appear to cross the placental barrier, but arrives in tissues shortly after birth *de novo*. There is a tendency for it to accumulate in lung with advancing age ($r=0.52$; $P<0.001$), but not in liver, kidney, aorta or intestine. Small but significant ($P<0.001$) increments were found in heart, prostate, uterus and trachea.

TABLE 1. TIN IN HUMAN TISSUES BY AGE, MEAN U.S. VALUES (p.p.m. ash)

Age group (yr)	0	0–1	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–84
Kidney										
Concentration	0	57*	60*	33	20	34*	29*	22	34	32
Occurrence	0/16	9/10	19/20	10/15	17/21	36/43	22/26	26/27	13/14	8/9
Liver										
Concentration	0	48*	61*	42	34	33*	25	35	36*	34*
Occurrence	0/21	11/12	22/23	14/14	20/21	39/43	23/28	25/25	13/13	9/9
Lung										
Concentration	(18)	35	34	45*	27	31*	39*	53*	58	64*
Occurrence	1/10	5/6	5/6	10/13	18/21	34/35	27/27	28/29	13/13	9/9
Ileum										
Concentration	0	101	98	80	174	116	97	53	172	140
Occurrence	0/8	1/2	1/1	9/10	12/13	26/28	16/17	12/12	6/6	3/4
Total occurrence (%)	1.8	86.7	94.0	82.7	88.2	79.8	91.9	97.8	97.7	93.5

Note: Concentrations of positive samples.
Occurrence refers to the number positive/total cases.
*Excluding values > 150.

Geographical variations in tissue tin

Tissues from 8 cities of the United States showed no marked variations in average organ tin. Mean concentrations in 24–27 samples of kidney from each location ranged from 0.23 to 0.76 p.p.m. wet weight; in liver from 0.35 to 1.0 p.p.m.; in lung

TABLE 2. GEOGRAPHICAL DISTRIBUTION OF TIN IN KIDNEY, LIVER AND LUNG OF MAN (p.p.m. ash)

	No. of samples	% present	Mean	Range	Median
KIDNEY					
United States	161	97	30	<5–480	20
Switzerland	9	33	5	<5– 17	<5
Africa	53	19	5	<5– 30	<5
Middle East	43	42	8	<5– 55	<5
Far East	57	40	11	<5–110	<5
LIVER					
United States	163	96	35	<5–300	24
Switzerland	9	78	6	<5– 20	Tc
Africa	49	25	4	<5– 20	<5
Middle East	44	55	11	<5–140	Tc
Far East	54	41	10	<5– 74	<5
LUNG					
United States	159	98	69	<5–920	39
Switzerland	7	100	37	23– 62	28
Africa	50	60	10	<5– 40	Tc
Middle East	45	91	33	<5–200	17
Far East	57	93	65	<5–1200	29

Note: Tc = trace, or barely detectable.
Mean and median values were obtained by assigning a value of one-half the least detectable amount to those cases where tin was not detected.

from 0.44 to 1.20 p.p.m. and in aorta from 0.22 to 0.94 p.p.m. No single area showed tin to be present infrequently and it was uniformly present in all four of these tissues from Seattle, San Francisco and Richmond, Virginia. Therefore, Americans in these cities appeared to be relatively uniformly exposed.

Table 2 shows the tin found in geographical groups from around the world. Tin in Africans was notably low. It was undetected in 75 per cent of livers, 81 per cent of kidneys, 40 per cent of lungs, 80 per cent of hearts, 76 per cent of spleens and 63 per cent of aortas. Tin was not found in any organ from a third of 51 subjects and was present only in lung in 30 other cases from Africa.

All geographical groups had lower mean and median values than Americans, differences being less marked in the case of lung. Detailed analyses of local samples revealed tin in one or more organs from each of 84 subjects from Japan, Manila, Hong Kong, Honolulu, Anchorage in Alaska, Beirut, Bombay, and Berne in Switzerland, although 11 had it only in lung. It was not detected in any organ from 8 of 49 subjects from Delhi, Lucknow, Vellore, Bangkok and Taipei, and was only present in lung in 6 others. Exposures to tin, therefore, appear to be a local phenomenon in both absolute and relative terms.

Despite the small number of cases, regional differences in organ tin were evident in Africa. Seven areas supplied subjects. Excluding lung, there was no tin detected in the kidneys, livers, hearts, aortas and spleens of natives living under more or less primitive conditions in Welkom, Usumbura and Kampala, a total of 71 analyses. Tin was found in 125 of these organs, 41 times in city dwellers of Cairo, Addis Ababa and Lagos. A small series from Lambarene, on the other hand, comprised of natives living under very primitive conditions, showed tin in 9 of 21 samples, suggesting that Gabon might be a tin-bearing area or that some subjects had had access to canned foods.

Even more marked geographical differences are evident when foreign and American concentrations are compared in terms of values falling within the lower third of the range for different tissues. For kidney, 89 per cent of Swiss, 91 per cent of Africans, 89 per cent of Middle Eastern and 74 per cent of Far Eastern values were within the lower third of the United States values. For liver, the percentage of values in the lower third were: Swiss 89, African 98, Middle Eastern 82 and Far Eastern 80; for lung: Swiss 29, African 90, Middle Eastern 60, Far Eastern 42. Therefore the data indicate that Africans are exposed to much less tin than are Americans, while people of other areas may collect it in their lungs but do not accumulate much in their kidneys and livers.

Because of the relative infrequency of atherosclerosis in many areas of Central Africa and its apparently increasing prevalence in Asia, Europe and America, individual values of aortic tin were correlated with the cause of death of 98 subjects from the United States and 68 subjects from the Far East, of whom 32 per cent and 28 per cent, respectively, died of atherosclerotic complications. No local or general correlations were found.

Tin in fresh and processed foods

Concentrations in vegetable foods grown in this area (Southern Vermont) are shown in Table 3. Unfortunately for the purpose of this survey, the soil of this area was found to contain considerable amounts of tin. This occurrence is unusual, for most soils examined in the United States are devoid of tin (see Discussion). The

TABLE 3. TIN IN FRESH AND PROCESSED FOOD AND TISSUES (wet weight)

Sample	μg/g	μg/100 cal	Source
Condiments			
Garlic bud	0.3	—	Chain store
Cloves, packaged	0.47	—	Chain store
Vegetables			
Peas, split, green, dried, bulk	1.92	64	Chain store
Peas, split, dried, packaged	8.50	281	Chain store
Peas, fresh	1.06	166	Home garden
Peas, fresh, fertilized	1.69	264	Home garden with phosphate
Beans, Navy, dried	5.80	191	Chain store
Beans, string, fresh	0.28	187	Home garden
Potato, raw	0.97	111	Vermont
Turnip, raw	0.11	61	Home garden
Turnip greens	0.07	64	Home garden
Radishes	0.38	253	Home garden
Radish leaves	0.0	—	Home garden
Parsnips	1.02	203	Institute garden
Swiss Chard	0.23	88	Institute garden
Lettuce, iceberg	0.07	61	Home garden
Spinach	1.23	473	Institute garden
Spinach	0.89	342	Home garden
Spinach, fresh, packaged	6.47	2488	Troy, N.Y.
Cabbage, raw	0.40	95	Institute garden
Kale	0.86	430	Home garden
Asparagus, fresh	9.07	5039	Local "organically" grown
Rhubarb, fresh	1.29	2150	Institute garden
Rhubarb, fresh	1.88	3133	Local "organically" grown
Mushrooms, fresh	1.08	1543	Chain store
Mushrooms, in glass	0.72	1029	Chain store
Soil, dry weight	161.94	—	Home garden
Soil, dry weight	32.71	—	Institute garden
Animal tissues			
Lamb liver	0.32	23	Vermont
Lamb chop	1.36	72	Local market
Beef, ground, lean	2.76	153	Local market
Beef, roast	0.51	23	Local market
Beef, brisket	1.10	28	Local market
Beef and fat, ground	3.44	90	Local market
Beef, bone marrow, forelimb	0.42	—	Chain store
Pork, lean chop	0.84	26	Local market
Chicken breast, frozen	1.73	92	Local market
Gelatin, packaged	3.50	135	Chain store
Lobster, claw meat	0.60	50	Maine
Oysters, frozen	1.38	276	Cape Cod
Halibut steak	1.21	121	Chain store
Codfish steak, 1	3.67	439	Chain store
Codfish steak, 2	0.49	60	Chain store
Greysole fillet	3.21	382	Newfoundland
Bloaters, smoked	4.30	169	Chain store
Deer liver	0.82	—	Vermont
Deer kidney	2.20	—	Vermont
Deer kidney	1.65	—	Vermont
Deer heart	0.0	—	Vermont
Deer kidneys (4)	0.0	—	Tennessee
Red fox liver	3.30	—	Vermont

TABLE 3. (*continued*)

Sample	μg/g	μg/100 cal	Source
Animal tissues (*continued*)			
Ruffed grouse liver	0.50	—	Vermont
Muskrat liver	0.33	—	Vermont
Woodchuck livers (3)	1.77	—	Vermont
Beaver heart	7.28	—	Vermont
Cereals and grains			
Rye, winter	1.9	57	Fallow ground
Soil, field, dry weight	37.10	—	Where rye grown
Rye flour from seed	0.0	—	Feed store
Barley	0.65	18	Feed store
Corn meal	0.11	3	Chicago
Corn, fresh on cob	0.12	4	Local store
Macaroni	4.76	418	Chain store
Wheat flour	0.47	14	Japan
Wheat flour	0.0	—	U.S.
Wheat seed	0.0	—	Feed store
Wheat bread	2.48	100	Chain store
Oats, seed	2.28	—	Feed store
Oatmeal, packaged	2.63	66	Chain store
Rice, packaged	0.28	8	Chain store
Dairy products			
Milk, dry skim, packaged	0.96	29	Nationally distributed
Milk, raw, bulk cans tinned	0.68	100	Local farm
Milk, from udder into polyethylene	0.19	29	Local farm
Cheese, tinfoil wrapped	0.32	8	France
Eggs, chicken	0.91	57	Local farm
Miscellaneous			
Peach	0.0	—	Chain store
Tea, packaged, bags	2.28	—	Ceylon
Rat diet (rye, corn oil, dry skim milk)	0.28	7	
Laboratory dog chow	1.0	25	Spectrographic method*
Dog meal	0.8	20	Spectrographic method*
Rabbit pellets	2.0	50	Spectrographic method
Rat diet, commercial	0.8	20	Spectrographic method
20% Superphosphate	3.24	—	Florida
Phosphate rock	4.24	—	Tennessee
20% Superphosphate†	4.06	—	Yugoslavia
Cigarettes	1.58	—	National brand
Urine, human	0.0	—	Normal female

*Data from R. G. KEENAN, Principal Spectroscopist, U.S.P.H.S. Occupational Health Field Hq., Cincinnati, Ohio.
Caloric values were calculated from MCCASKEY, R. A. and WINDOWSKI, E. M. *The Chemical Composition of Foods*, 1947, Chemical Pub. Co., Brooklyn, N.Y.
†Furnished by R. C. GRIGGS.

analyses of fresh foods are therefore meaningful only in relation to this area and cannot be construed as universally applicable. All fresh vegetables showed tin, usually less than 1 p.p.m. Rye grown on a fallow forest field absorbed tin. Local asparagus had large amounts. Vegetables imported into this area also usually contained much tin, especially dried legumes, grains and packaged spinach, although precautions undoubtedly were not taken to avoid contamination during washing and

TABLE 4. TIN IN CANNED FOOD AND IN WATER PIPES (wet weight)

Sample	μg/g	μg/100 cal	Source
Coated cans			
Condensed tomato soup	7.35	1470	Chain store
Green beans, French	2.84	4055	Chain store
Whole beets	3.38	768	Chain store
Carrots, dietetic	6.45	3071	Cleveland, Ohio
Apricots, sweetened	4.99	818	Cleveland, Ohio
Spinach, dietetic	11.82	4546	Cleveland, Ohio
Asparagus	8.96	4922	California
Uncoated cans			
Kipper snacks	61.88	3094	Norway
Anchovies	129.31	5851	Portugal
Sardines	18.65	634	Morocco
Sardines	1.57	53	Portugal
Corn oil, bulk	4.10	45	National brand
Coffee, ground	3.65	—	National brand
Molasses	4.15	161	Louisiana
Chili powder	3.9	—	Chain store
Thyme, ground	4.8	—	Chain store
Cloves, whole	7.26	—	Chain store
Water pipes			
Iron pipe	53.28	—	Dissolved in acid
Copper pipe	53.40	—	Dissolved in acid

packing. The accumulation of tin by milk from udder to table undoubtedly represents contamination. The finding of large amounts of tin in superphosphates suggests a possible source from fertilizers.*

Tin in canned foods

Table 4 shows values for tin in canned foods. Most modern cans are lacquered, which reduces contact of food with tin. In almost every case, however, concentrations were larger than those in fresh foods, often providing several mg in 100 calories. The high contents in three oily fish in unlacquered cans are noteworthy.

Tin in animal tissues

The few specimens from local wild animals contained tin (Table 3), as might be expected from its presence in soil and local vegetation. It was not detected, however, by spectrographic methods in the kidneys of four Tennessee deer, probably indicating that vegetation in that area contained little. Food of animal origin always showed tin, especially large amounts being found in some sea foods. There was some variation between samples of the same tissue, which may have come from contamination.

Tin in a sample diet

A one-day institutional diet contained 3.6 mg of tin (Table 5). It is likely that the

*The 8 foods grown in the home unfertilized garden contained a mean of 0.47 μg/g, the 6 grown in the fertilized institute garden had 0.86 μg/g, while the 5 obtained from chain stores (corrected for wet weight) had 1.56 μg/g. There was an increase in tin when peas and spinach were grown on fertilized soil and the spinach packaged. Absorption from soil may depend, as it does for other metals, upon soil pH and not directly upon concentration of metal in soil.

TABLE 5. TIN IN INSTITUTIONAL DIET

	Wet wt (g)	% dry substance	μg/g wet	μg/g dry	Total μg
Breakfast	766.0	25.8	1.24	4.86	949.8
Dinner	860.8	18.4	1.65	8.97	1420.6
Supper	898.7	19.1	1.32	6.91	1186.3
Total or mean	2525.5	21.1	1.41	6.78	3556.7

Note: *Breakfast*: canned grapefruit juice, oatmeal, griddle cakes and syrup, toast, butter, coffee.
Dinner: roast pork and gravy, mashed potatoes, canned sauerkraut, bread, butter, canned applesauce, coffee.
Supper: baked macaroni and cheese, canned orange and grapefruit with bananas on lettuce, jello, bread, butter, tea.

major portion came from canned fruit juices and fruits, for the mean concentration of 1.41 μg/g was seldom exceeded by those of the other foods and was usually less.

TABLE 6. TIN IN VEGETATION FROM SOUTHERN VERMONT (μg/g)

Sample	Wet	Dry	Ash
Pine, white, needles *Pinus strobus*	0.37†	0.90	23.1
Spruce, red, needles *Picea rubra*	0.0†	—	—
Soft wood chips	0.70	0.79	175.0
Elm, section, 40 yr old	1.70	1.79	255.0
Elm, section, 80 yr old	1.39	1.47	154.0
Clover, *Trifolium pratense*	0.38*	2.11	13.3
Wild blueberries	0.14	1.02	14.6
Poplar leaves, *Populus tremuloides*	0.0*	—	—
Maple leaves, *Acer saccharum*	0.18*	0.53	9.8
Birch leaves, *Betula alba*	0.0	—	—
Oak leaves, *Quercus rubra*	0.0*	—	—
Alfalfa, *Medicago sativum*	2.92*	13.27	161.1
Fern, suburban, fronds	0.69†	4.60	27.6
Peonies, pink flowers	0.0	—	—
Barberries	1.06	4.24	89.2
Mountain ash, berries	0.10	0.50	11.1
Soil, forest	—	156.70	—

*Not detected in Finland, according to LOUNAMAA [17].
†Occasionally detected.
Note: Trees and blueberries were situated in a remote hilltop forest; the elm, fern and mountain ash were suburban. The clover and alfalfa came from a pasture 5 miles from a town.

Tin in vegetation

Analytical results for a few samples of local vegetation are shown in Table 6. The absorption by plants was erratic, although soil tin was high. This unexpected phenomenon limits the general significance of our survey. Tin in sections of an 100-year old elm indicates that exposure of this tree is not of recent origin and that the subsoil has contained the metal.

Intake of man

From these analyses, diets low and high in tin can be formulated. If 800 calories came from 7 canned vegetables and 1 canned fish and the remainder of the diet

contained no tin, 23 mg would be ingested. If the remainder included 600 calories of dried peas, packaged spinach, fresh asparagus, mushrooms, rhubarb, and certain fish, another 14 mg would be added. Making up a remaining 1000 calories from grain products and milk would add less than 1 mg to give a total of 38 mg per day, certainly a large amount for a trace metal. On the other hand, a diet of 2400 calories composed largely of some fresh meats, grain products and vegetables would contain about 1 mg. Therefore, human intake may vary considerably.

DISCUSSION

Geochemistry of tin

MASON says: "For an element to have biogeochemical significance, it must either be shown to participate in the life of the organism or else to be present consistently in concentrations greater than that in the environment" [14].

Of the 30 trace metals of possible or probable biological importance, tin ranks 21st in the universe, 17th on the geosphere (3 p.p.m.), 12th in the hydrosphere (3 p.p.b.) and 8th in the body of American man (0.4 p.p.m.); thus abundance has progressively increased. This fact on the surface is somewhat surprising, for the ubiquity of tin on this planet has not been demonstrated, vast areas in the northern latitudes apparently lacking it. Tin is one metal, according to GOLDSCHMIDT [15], which unquestionably differs in abundance in various regions of the globe and is frequently undetectable as a trace element in magmatic rocks. Extensive data compiled by the United States Geological Survey [16] failed to show detectable tin in marine evaporites (except those in Germany), in volcanic emanations (except from a single source in Japan), in biogenic and chemogenic siliceous sediments and in sandstones from many areas of the world.

The extensive investigations of LOUNAMAA [17] on the uptake of trace metals by plants in Finland included tin. He found it only in soils overlying silicic outcrops, and then relatively seldom, the highest concentrations varying from 10 to 30 p.p.m. The mean concentration in the one outcrop of silicic rock where it was present was 3.8 ± 0.56 p.p.m. and in overlying silicic soils $4.5 + 0.5$ p.p.m. (Table 7). MITCHELL [18] also found less than 10 p.p.m. in English soils, although podzol granite soils of Western Moravia contain 500–1000 p.p.m. ALLISON and GAUDIUM [19] did not detect tin in 54 soil samples from Florida, nor in 52 from other states, by spectrographic methods. CARRIGAN and ROGERS [20] found no tin in Florida soil or in plants grown on it. Tin has been mined in small amounts in only 11 states in this country. Therefore tin does not appear to be ubiquitous on the geosphere but occurs in localized areas such as ours, to which it was probably carried from the North by glacial movements. If so, we should expect tin deposits in Eastern Canada.*

CLARKE [21], the father of geochemistry, stated in 1924: "Tin is one of the rarer metals and its ores are not numerous. Native tin is occasionally found, but never in more than trifling quantities . . . , in association with granite rocks." He discusses the known deposits in Alaska, Mexico, Nevada, Texas, Virginia, California, Maine, South

*This hypothesis was confirmed by H. V. WARREN (personal communication): A mining company is . . . "developing what we hope will turn out to be an important tin-producing property in New Brunswick, in a district which might be regarded as an extension of the one in which you are working." He also states that unmineralized areas in Devon and Cornwall have 75 p.p.m. normally in soil, and mineralized areas up to and more than 1000 p.p.m. According to C. G. DOLL, State Geologist, "Tin has not been recorded in Vermont geological literature" (personal communication).

TABLE 7. TIN IN PLANTS (After LOUNAMAA [17])

Species	No. of samples	Positive samples		Soil*	Content (p.p.m. ash)	
		No.	%		Mean ± S.E.M.	Range
Lichens	59	55	93.2	S	72±4.7	<10–100
				U	37±8.0	10– 60
Mosses	16	10	62.5	S	24±8.6	<10– 60
				U	18±5.5	<10– 30
				C	12±9.0	<10– 30
Ferns, fronds	93	20	21.5	S	3.2±0.21	<10– 10
Conifers	83	9	10.8	S	3.2±0.15	<10– 10
Deciduous trees and shrubs	117	2†	1.7			<10– 30
Dwarf shrubs	48	5	10.4	U	6.0±3.0	<10– 30
Herbs	403	7	1.7	U	3.8±0.80	<10– 30
Grasses	30	1	3.3		300	—
Soil	94	13	13.8	S	4.5±0.50	<10– 30
	23	0	0	U	—	<10
	17	3	17.6	C	4.6±1.6	<10– 30
Rock	180	8	4.4	S	3.8±0.56	<10–100
	50	0	0	U	—	<10
	34	0	0	C	—	<10

*S=silicic, U=ultrabasic, C=calcareous. These terms describe the character of the underlying rocks from which the soil was formed.

†The birch *Betula verrucosa* was the only deciduous tree or shrub containing tin, 2 of 59 samples showing it. Of the dwarf shrubs, 2 of 13 samples of mountain cranberry, 2 of 16 samples of heather and 1 of 4 samples of crowberry contained it. Two of 26 Norway Spruce and 7 of 43 white pine likewise contained this metal. In no species of herb was more than one sample positive. Our small survey (Table 6) differs markedly because of tin in our soil.

Dakota, Cornwall, Tasmania, the Malay Peninsula, Saxony, South Africa and Bolivia. To these must be added the Belgian Congo, Indonesia, Thailand, China, U.S.S.R. and Nigeria [2].

Tin in sea water and marine organisms

In sea water, tin is present in amounts of about 3 p.p.b. according to VINOGRADOV [22] and MASON [14]. Therefore, its presence in marine organisms is to be expected, although little attention has been paid to it. Tin has been found in marine algae but was not detected in plankton, bacteria, flowering plants, protozoa, sponges (although BOWEN and SUTTON [23] found it accumulating in one sponge), coelenterates, echinoderms, crustacea, or most fishes; small amounts were found in a marine worm, an oyster and a dogfish [22]. It would appear that most marine organisms have an aversion to tin and that little enters the body of man via seafoods, although we found it constantly. The presence of tin in phosphate fertilizers which come from marine phosphorites, suggests that marine animals in the Pleistocene era absorbed it from the sea.

Tin in fresh water

No tin was detected in 119 analyses at 71 locations on 28 major rivers of the United States by the National Water Quality Network [24]. DURUM and HAFFTY of the Geological Survey found tin in only 3 of 59 samples from 15 major North American rivers, the Apalachicola (Florida), the Mobile (Alabama) and the Nelson (Manitoba) [25]. Maine waters contained little tin, mean 0.038 p.p.b. with a high of 2.50 p.p.b. It was not reported in any other river, lake or sub-surface waters of the world [16]. Many salts of tin are insoluble and it is unlikely that factory effluents contribute much to rivers.

There is less agreement on municipal waters. DURUM and HAFFTY of the Geological Survey [26] analyzed supplies from 42 United States cities; tin was detected in only two (1.1 and 2.2 p.p.b.). On the other hand, analyses by the United States Public Health Service showed concentrations of 0.8–30 p.p.b. in 32 of 175 finished municipal waters [27]. It is known that corrosive waters when acidic or alkaline can attack bronze fittings in pumping stations, presumably releasing some tin, but correlations of corrosion and water tin have not been made. Obviously, very little tin enters the body of man from water.

Tin in air

Sizeable amounts of tin occur in the air of American cities, especially where much industry is located. Amounts from 0.003 to 0.3 μg/m³ were found in 60.6 per cent of 754 samples taken from 22 cities [28]. Suburban areas had least, while 17 per cent of 312 samples from 6 major industrial centers had 0.03–0.3 μg/m³. Assuming a respiratory volume of 20 m³/day, 740 μg of tin in the lungs, and deposition of one-eighth of the tin inspired, air containing 0.015 μg/m³ would not account for human pulmonary levels until age 55. Presumably some pulmonary tin in American adults comes from air, however, for many foreigners had little.

Atomic structure and chemical activity

Tin occurs in three valence states, the trivalent form being unstable. Most of its basic properties lie between germanium and lead, the other two members of its group in the Periodic Table. The ionic radius of tetravalent tin is close to those of nickel, cobaltous and molybdenum (IV) ions. Divalent tin is readily oxidized in solution.

Tin differs considerably from lead in many of its chemical reactions, but resembles germanium quite closely. Complexes of stannous hexachloride with co-ordination number 6 have a condensed structure of octahedra sharing edges [29]. Other chelates are formed with difficulty, and such condensed structures may not be applicable to biomaterial. Tin, however, readily forms lakes with anthocyanin and other pigments* and combines with halogens, dithiols, and proteins, the last becoming insoluble.

Tin in plants

GOLDSCHMIDT [15] found tin enriched in forest litter and humus and concentrated in coal. Samples were presumably taken in Germany which has tin deposits. GORDON [31] reported 30–300 p.p.m. in Finnish peat. HANNA and GRANT [32] found tin

*For those interested in kitchen chemistry, a simple test for tin is available. The juice of black currants or blackberries turns blue or violet in the presence of tin. Iron gives a similar color. The juice of Persian berries turns yellow, a pigment used in confectionery [30].

present in all but 7 of 43 samples of foliage from 13 species of ornamental plants and trees in New Jersey. Species variations occurred in plants grown on the same soil and no effect of soil pH was found. The usual concentrations were less than 0.5 p.p.m. dry weight.

LOUNAMAA [17], studying wild Finnish plants, found that lichens concentrated tin to exceptionally high levels considering the small amounts in rocks. Those growing on silicic rocks had 72±4.7 p.p.m. (range <10–100) and those on ultrabasic rocks 37±8.0 (range 10–60). Six of 16 mosses had undetectable amounts, the remainder showing 10–30 p.p.m. Tin in higher plants was present only sporadically (Table 7). His limit of detection was 10 p.p.m. ash. Tin therefore may be accumulated by primitive plants against an environmental gradient, but in Finland, at least, does not ordinarily enter other vegetation from soil.

The presence of tin in wild plants may be an entirely local phenomenon dependent upon whether or not the plant comes from tin-bearing areas. MITCHELL [18] found it in English cultivated beets, hay, grain and pasture herbage. On the other hand, a series of studies on Spanish vegetable foods by LOPEZ DE AZCONA, SANTOS RUIZ and DEAN GUELBENZO [33] by spectrographic methods failed to detect tin in cereals, legumes, grains, condiments, spices, coffee, garden vegetables and fruit, except when canned. Apparently some areas of Spain, like Finland, are relatively devoid of tin.

ALLISON and WHITEHEAD [34] found tin in some American organic fertilizers, such as castor, peanut, soybean and cottonseed meals. HODGKISS and ERRINGTON [35] found it in Kentucky hay and THOROLD [36] in African coffee wood. HUNTER [37] detected it in American bracken, and NAGATA [38] in Japanese tea. Otherwise reports are meager and there is little data on fresh foods.

At this point we can draw certain conclusions from the data as presented. Tin is not ubiquitous. Therefore it is not an essential element for plants. Because it is not always found in plants, it is presumably not essential for mammals. Because it is not found in most marine organisms, it is presumably not essential for marine life. For an element to perform a necessary biological function, it must be uniformly available, or living things would have remained localized to areas where it was present. If sodium, for example, were not ubiquitous, the development of terrestrial animals would have been confined to the littoral, and evolution of vertebrates probably would have ceased at the amphibian stage.

The difference between tin in American and foreign human tissues, and its absence in many African subjects further indicates that it is not essential for man. Therefore we must consider sources of supply as coming usually from other than the natural environment.

Tin in mammalian tissues

The occurrence of tin was surveyed by KEHOE *et al.* in 1940 [39] by spectrochemical methods; they found it in about 80 per cent of samples of all normal human tissues, blood and urine. There appeared to be an approximate balance between amounts excreted in feces and ingested in food. When present in blood (mean value 14 μg/100 g)* almost all was in erythrocytes (mean 11 μg/100 g). Successive weekly samples

*By X-ray emission spectroscopy. GUFMAN *et al.* found serum tin in 22 subjects to be 0.0±0.93 μg/ml (mean ±s.d.) [60]. The highest value was 1.22 μg/ml, or about 5 times KEHOE's upper limit.

from a single male varied from <5 μg to 25 μg. Brain showed little, as we also have found.

Prior to their analyses, tin had been demonstrated in human tissues [40] and in almost all tissues of the ox, horse and sheep, at concentrations of 0.5–4.0 p.p.m. dry weight. It has been found in human teeth [41], in spinal fluid [42] and in human milk and cow's processed milk [43]. There is little data on fresh foods [30].

Biological effects

There is remarkably little in the literature on the action of tin on plants and animals, considering its wide use. COHEN [44] found that traces stimulated root growth in the sunflower, while 5 p.p.m. were toxic to it and to corn and peas. MICHIELS [45] demonstrated stimulatory actions of colloidal tin on a number of grains and legumes, while tomatoes grown on peat seemed to benefit. The evidence is far from convincing. No positive actions by traces of tin on bacteria, small mammals or man have been demonstrated to our knowledge, although the literature of half a century ago contains references to its lack of effect on laboratory animals.

Toxicity of tin

Relatively large doses of tin can be tolerated orally by mammals. Cats and rats have taken 30–50 mg/kg of body weight per day; dogs and man 850–1000 mg/day [30]. Enormous doses, such as 1 g every 6 or 10 days given to rabbits caused death in one or two months with renal and hepatic degeneration and paralysis of the hind legs. The fatal dose of stannous chloride is given as 4–6 g. Severe gastrointestinal symptoms in man have resulted from a fruit punch (pH 3) containing 2 g/l. absorbed from a retinned 5-gal milk can [46]. Goldfish have survived 1000 p.p.m. in water for 1–1½ hr, but were killed by 626 p.p.m. in 4.5 hr; *Daphnia magna* were killed by tetravalent tin at 146 p.p.m. and by divalent tin at 25 p.p.m., in the low range of the toxicity of barium, antimony and manganese. Young eels, however, succumbed at 6 p.p.m. in less than 3 hr. Sand cultures containing 40 p.p.m. stannous ion did not affect the growth of sugar beets [47]. Tin, therefore, is one of the least toxic of the heavy metals in terms of overt effects.

SOLLMANN [48] discusses the pharmacology of tin briefly, stating that small quantities accumulate in the tissues. Injected tin caused marked injury to the kidneys, but its acute toxicity is low. Older observers reported chronic effects, ranging from gastroenteritis to ataxia and motor paralyses, resembling chronic lead poisoning. Inhaled tetramethyl and tetraethyl tin caused aliphatic narcosis, followed by a temporary paralysis and delayed encephalopathy.

It is interesting, considering the apparent neurotoxicity of inhaled and injected tin, that the human brain was usually devoid of it. Only 5.5 per cent of 75 brains analyzed showed tin, always in trace amounts (<5 p.p.m. ash). Apparently it seldom crosses the 'blood–brain barrier,' except, perhaps, in organic forms, possibly because of lack of affinity for lipids. VARDANIS and QUASTEL [49] have offered an explanation for the effects of tetraethyl tin (and lead) on cerebral cortex. When rats were exposed to small concentrations causing symptoms, active transport of amino acids and oxidation of glutamate by slices of their brains were inhibited, without affecting glucose metabolism. In addition, potassium-stimulated cerebral respiration was abolished. These authors suggest that the compounds act on phospholipid groups concerned with

amino acid and cation transport at the cell membrane. CREMER, however, demonstrated that tetraethyl metal compounds were rapidly converted to triethyl tin and lead by the liver, these substances being extremely active inhibitors of metabolic processes, particularly of cerebral glucose metabolism [50]. Triethyl tin is a potent uncoupler of oxidative phosphorylation, as are several other trace metals.

The nephrotoxicity of injected tin was demonstrated by HADDON [51] for the divalent metal chloride. Lower nephron necrosis caused death in all rats at 65 mg; the LD_{50} at 72 hr being 32.5 mg. One-tenth of this dose given at 3-day intervals for 20 doses either produced no changes or caused a variety of renal lesions. This LD_{50} dose for the rat is equal to the total content of the American 'Standard Man.' Therefore, while ingested tin usually does not appear to be toxic, injected tin in comparatively large amounts is quite nephrotoxic, like other heavy metals. Recondite toxicity for the lifetime of animals has not been ascertained but is being investigated. No local deleterious effects on gingival tissue have been observed [52].

Tin in canned food

An enormous amount of tin can accumulate in foods in contact with tin-plate. In 1908 a suggested limit for canned food was set at 286 p.p.m. MONIER-WILLIAMS [30] discusses the subject at length, both regarding double-lacquered cans, where the content rarely exceeded 40 p.p.m., and plain cans, where it was usually below 100 p.p.m. Larger amounts have been found (p.p.m.): 300 in asparagus, 680 in spinach, 700 in apricots, 760 in fish oil and 2500 in anchovies in tomato sauce. Foods preserved in heavily tinned cans from the time of Parry's Arctic Expedition in 1824 had up to 2400 p.p.m.

Modern cans have two improvements which decrease exposures of food to tin. The cans are lacquered. The tops are crimped on, allowing little contact of foods with solder (tin, 2–4 per cent, the remainder lead; occasionally tin up to 30 per cent). Imported cans, containing such foods as anchovies, sardines and patés, often show no lacquer and food may be in direct contact with solder. Lacquers, however, may develop pinholes in time, allowing contact of food with tin and starting corrosion. Therefore, storage of canned food can result in accumulation of tin.

Any condition which favors the removal of stannous ions from solution influences the absorption of tin from cans, according to MONIER-WILLIAMS [30]. Corrosion is increased by air, which promotes attack of tin by acids. Chelation of tin by organic acids allows more stannous ions from the lining to go into solution, tin becoming anodic to iron (pinholes in tin plating invariably occur). Oxidizing agents, air, ferric salts, nitrates, cupric salts, certain pigments, sulfur dioxide and other sulfur compounds promote corrosion. Other substances are oils, acid fruits and salt. Cheese wrapped in tinfoil may be attacked by lactic and citric acids; from 300–2000 p.p.m. have been found, the larger values after prolonged contact. Upon exposure to air and rewrapping a cheese, tin increased from 300 to 2000 p.p.m. [30].*

One cannot help but wonder whether or not exposures of civilized man to tin have decreased with the use of lacquers and modern methods of sealing cans, while exposures of primitive man have increased from very low levels with accessibility to canned food. If the analytical data are correct, American food contains less tin than

*SOLOV'EVA showed that vegetables in lacquered cans absorbed tin and iron in proportion to the length of storage from 12 to 18 months, to an amount of < 100 p.p.m. [61].

formerly. BUCHANAN and SCHRYVER [53] in 1908 suggested 286 p.p.m. as the allowable limit. ADAM and HORNER [54] in 1937 found most tinned foods had less than 100 p.p.m. and those in lacquered cans less than 40 p.p.m. Most of 200 samples of canned marine foods marketed in the Orient in 1962, however, contained more than 150 p.p.m. tin, especially when stored over 12 months [55]; the cans were unlacquered. In 1940 KEHOE *et al.* measured the dietary intake of normal man as 17.14 ± 10.17 mg (mean ± S.D.), a figure which would give about 7 p.p.m. in a 2500 g diet and 14 p.p.m. if half of the food was canned and the other half contained no tin. Our analyses show a fifth of this value in a sample institutional diet and few of our fresh or canned foods contained 7 p.p.m. tin.

A new exposure involves the use of stannous fluoride in dentrifices. Extrapolation of increasing use during the past few years indicates that it will be widespread in a few more years, to a small extent replacing previous exposures due to solders and unlacquered cans.

Balance in man

PERRY and PERRY [56] found that 24 adult subjects in St. Louis excreted 16.6 ± 1.84 μg/l. of tin in urine (range 3–40 μg/l.), or 23.4 μg/day. This amount is slightly more than one-fifteen hundredth (0.06%) of our calculated intake. Tin was found in every sample and there were no differences between the sexes or between the two races. A third of the samples from African patients, however, did not contain any detectable tin. A single careful experiment showed that urinary tin was doubled in mild alkalosis and depressed in acidosis; the total excreted was also more than doubled in high volumes of urine. The Perrys' two sons excreted less tin than did their grandfathers. KEHOE *et al.* [39] found 18 ± 2 μg/l. in 30 American urine samples, 9 ± 1 μg/l. in 30 Mexican urine samples and none in 34 French urine samples.

They measured tin in the food, fluids and feces of a normal American man for 28 days, with these results (mean/day ± S.E.M.):

Intake (mg)		*Output* (mg)	
Food and beverages	17.14 ± 1.92	Feces	22.88 ± 1.94
		Urine/l.	0.014 ± 0.001

Because exposures have decreased in the last 20 years, daily adult human balance of tin probably can now be approximated somewhat as follows:

Intake (mg)		*Output* (mg)	
Food	4.0 (range, 1–40)	Feces (estimated)	3.98 (range, 1–40)
Water	0.0 (range, 0–0.03)	Urine	0.023 (range, 0–0.04)
Air	0.003 (range, 0–0.007)		
Total	4.003 (1–40)		4.003 (1–40)

Retention of about 5.5 μg/day for the first 15 years of life would account for the accumulated bodily content of 30 mg. These balances, which are quite impressive in the light of the absence of steady accumulation (Table 1) suggest either that intestinal

transport and barrier systems are present in man, or that over 99 per cent of ingested tin is insoluble.*

Is tin an essential trace element?

Although no one has suggested seriously that tin might be essential, we must examine the evidence nevertheless, according to the several criteria by which we suspect a metal of having a biological function. Tin does not belong to this category.

1. Tin apparently is not ubiquitous on the earth's surface. Essential trace elements must be available.
2. Tin is not consistently found in plants, the source of trace metals for all animal life, directly and indirectly.† Its presence in animals probably is the result of sporadic exposures. In man, the evidence is convincing that measureable tin is not necessary for life or health.
3. The chemical nature of tin indicates that it does not act as a good catalyst in biological ranges of hydrogen ions.
4. Known biological effects, either stimulatory or depressant, are far from proven.
5. The newborn human being usually contains no detectable tin, although it is well supplied with essential trace metals.
6. Tin accumulates rapidly in tissues following environmental exposures, although its concentration increases little or none with age. Essential trace metals show little tendency to accumulate.

In only two respects does tin behave like an essential trace metal: (1) It has a low order of toxicity to living things. (2) The absence of progressive accumulation in most organs suggests either that homeostatic mechanisms are active in man or that ingested tin is largely insoluble in the gastrointestinal tract.

We can consider tin, therefore, as an 'abnormal' trace metal recently put in man's tissues as a result of his own inventiveness and his ability to adapt the environment to his needs. Because of its ubiquity in the tissues of civilized man, we must make efforts to prove that tin is completely without serious biological effects during a lifetime of exposure, and that recondite metabolic alterations of stannic origin may not initiate one or more degenerative disorders common to civilization. Lifetime studies in small mammals, such as we are conducting [58, 59], may help partly to answer this question. To date we have observed no effects of small doses of tin on the growth of weanling mice.

SUMMARY AND CONCLUSIONS

A survey of tin in the tissues of man from various areas of the world and of sources and exposures thereto has been made by spectrographic and microanalytical chemical methods.

Tin is not ubiquitious on the earth's crust. Mammalian exposures come from vegetation grown on more or less isolated tin-bearing areas (of which Southern Vermont

*The balances here given are at great variance with those of KEHR and MCCANCE [37] who found 50-90 per cent of tin ingested from food excreted in the urine. The differences are unresolved.

†In a list of 207 plants and trees for biogeochemical prospecting, compiled by CARLISLE and CLEVELAND [62], only heather, *Calluna vulgaris*, was given as containing tin. These authors undoubtedly referred to LOUSSAERT [17] who found it, however, in only 2 of 16 samples from one outcrop of tin-bearing soil. The rarity of tin in their list and its presence in some of our samples are further evidence of the scarcity of this element on the earth's crust, except for local areas.

is one). Human exposures come largely from canned food. Coated (lacquered) cans give up less tin to food than do uncoated cans. The human daily intake of tin has decreased during the past 20 years, and now approximates 4 mg.

Human tissues from civilized areas contain much more tin than do those from uncivilized areas. Adult human beings from the latter may be devoid of detectable tin.

Tin is seldom present in the newborn American. It is deposited in tissues rapidly during the first decade of life but does not accumulate with age. It has a predilection for the gastrointestinal tract, the lung, liver, kidney and aorta. Wide variations in tissue concentrations occur, presumably due to variations in exposures.

Overt toxicity is low, but little is known of recondite or sub-clinical toxicity.

Tin does not behave like an essential trace metal, but is an environmental contaminant largely the result of the preservation of food in cans.

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Arsenic, Germanium, Tin and Vanadium in Mice: Effects on growth, survival and tissue levels [1]

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ABSTRACT Certain trace elements found in the human environment may produce adverse biological effects when fed in small amounts to rats and mice for their lifetime. Others may be inert or have favorable actions. Mice numbering 643 were fed a diet of rye, corn oil and dried skim milk containing small amounts of arsenic, germanium and tin and larger amounts of vanadium, in an environment designed to exclude trace contaminants. Groups of 52 to 108 or more divided as to sex were given 5 ppm of these elements as arsenite, germanate, vanadyl or stannous ions in drinking water from weaning until natural death. Chromium (1 ppm) was added to the water of those given arsenic and germanium and their controls. No element but chromium affected growth. Median life spans and longevity of the oldest 10% of males given germanium and of both sexes given arsenic were somewhat shortened. This female toxicity was not observed in animals given tin and vanadium. All elements accumulated in one or more organs, germanium and tin in spleen with age. No element was carcinogenic. Life-term exposures of animals to trace elements may show inherent toxicities at levels tolerable to young animals.

Certain biological effects of small doses of several trace elements, given in drinking water to mice and rats from the time of weaning until death in an environment relatively free of contaminating metals, are under investigation. This report concerns one trace metal which may be introduced as a contaminant in food, tin, and three trace elements ordinarily present in most foods, arsenic, vanadium and germanium.

METHODS

The experimental conditions of the environment, and the food and water to which mice were exposed for their lifetimes have been reported in the first paper of this series (1) and have not been altered. In brief, the diet consisted of whole rye flour (60%), powdered skim milk (30%) and corn oil (9%), to which was added 1% iodized sodium chloride, 100 mg iron as ferrous sulfate and a vitamin mixture. The drinking water came from a mountain spring and was doubly deionized; to it were added the essential trace metals, zinc (50 μg/ml), copper (5 μg/ml), manganese (10 μg/ml), cobalt (1 μg/ml) and molybdenum (1 μg/ml) as nitrates, acetates or molybdate; these concentrations are similar to those present in commercial diets. Extensive precautions were taken to avoid metallic contamination (1).

Random-bred mice of the Charles River CD strain were born of pregnant females purchased from the supplier.[3] At the time of weaning 54 males and 54 females, 6/cage, were given the basic drinking water containing 5 μg/ml arsenic as sodium arsenite and 1 μg/ml chromium as chromic acetate; we have found chromium (III) to be a growth factor for rats and mice (1, 2). Another group of 71 male and 65 female weanling mice were given the water with added chromium and 5 μg/ml germanium as sodium germanate. Controls were 54 males and 54 females given the basic water with added chromium. Litters were divided among the 3 groups. The diet contained 0.46 μg/g arsenic and 0.32 μg/g germanium on a wet-weight basis.

At the time studies on tin and vanadium were begun, we were not fully aware of the essentially of chromium (III); there-

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fore this element was omitted from the drinking water. Mice of the same strain numbering 54 males and 54 females were given the basic water to which were added 5 μg/ml tin as stannous chloride, and 62.5 μg/ml ascorbic acid (to inhibit oxidation to stannic tin). Controls were 34 males and 46 females. The diet contained 0.28 μg/g tin, wet weight.

A pilot study on vanadium was made, using 23 males and 29 female mice which were given 5 μg/ml vanadium as vanadyl sulfate in the drinking water. We attempted to make a diet low in vanadium, and for this purpose used defatted fish flour[4] (30%), refined wheat flour (50%), white sugar (10%) and lard (10%), with sodium chloride (1%) and vitamins added. This diet was fed to mice for 6 months. It contained 1.4 μg/g vanadium. At the end of this period, the mice were given the regular diet, which contained 3.2 μg/g vanadium, on a wet basis. A similar number of controls, 25 males and 26 females, were treated identically, without ad. vanadium.

Animals were weighed weekly at [illegible] and then at monthly intervals. Dead animals were autopsied, tumors and gross lesions noted and abnormal tissues stored for microscopic analysis. Hearts, livers, kidneys, livers and spleens were pooled samples of 2 to 14 from various age[illegible] and analyzed for the elements given. Because of the small size of the samples, it was impossible to analyze all control tissues for each of the 4 elements.

Tissues analyzed for tin and vanadium were ashed at 450° in muffle furnace [illegible] handled as reported (3, 4). In the case of arsenic and germanium, they were ashed in a low-temperature asher[5] at approximately 105° in nascent oxygen under a partial vacuum (5, 6). Analytical methods used were: tin, the chemical method of Thompson and McClelland (3, 7).

[4] Vitalin Corporation, Monticello, Illinois.
[5] Tracerlab 500 A, Ashland, California.

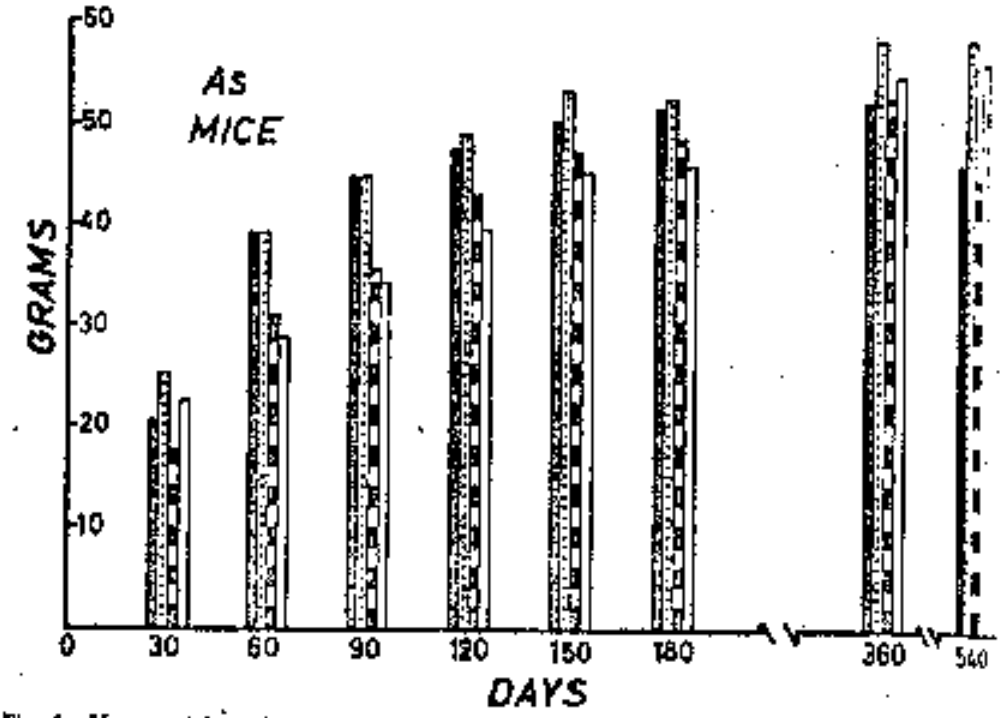

Fig. 1 Mean weights of mice given arsenic as arsenite at various ages. The solid bars represent males receiving arsenic, the dotted bars male controls. The dashed bars represent females [illegible] arsenic, the open bars their controls. Significant differences in weight were confined to the males. At 360 days, male controls exceeded arsenic-fed by 4.7 g ($P < 0.05$) and at 540 days 8.9 g ($P < 0.005$). The difference at 540 days in the female group was 7.9 g but was not statistically significant.

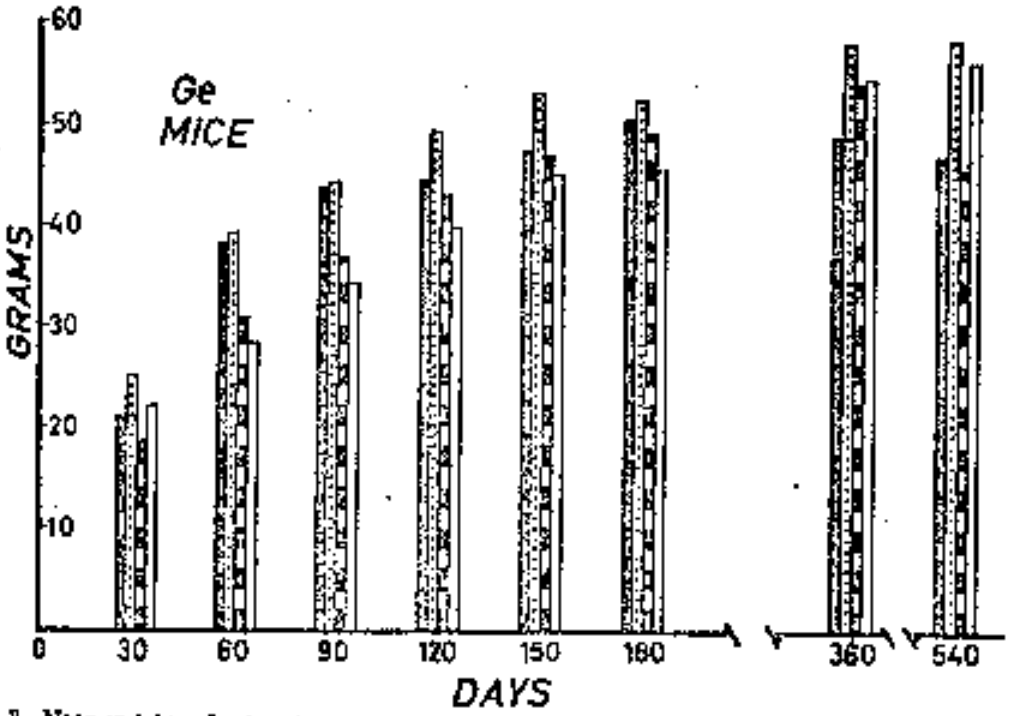

Fig. 2. Mean weights of mice given germanium (Ge) germanate at various ages. The solid bars represent males receiving germanium, the dotted bars male controls. The dashed bars represent females receiving germanium, the open bars their controls. Significant differences in weight were confined to males. Males at 360 days differed by 9.1 g ($P < 0.005$) and at 540 days by 8.7 g ($P < 0.025$). Females differed at 540 days by 8.9 g ($P < 0.005$).

...nium, that of Luke and Campbell (6, ...); arsenic, that of Sandell (5, 9), modified by that of Evans and Bandemer (10); vanadium, that of Sandell (11), slightly modified (4). Sensitivities and limits of detection of these methods in our laboratory have been reported (3–6).

RESULTS

Growth rates. None of the four trace metals significantly affected growth rates of the mice, compared with their controls. There were significant differences, however, in those given chromium and those given the former groups being heavier at ages ($P < 0.01$–0.005), as reported in other series of mice (1) and rats (2). Figures 1–4 show the respective mean weights. At 18 months of age body weight increased in females given each metal and in males given arsenic. Mice fed the fish-meal diet gained 29.3 g and those fed the same diet with added vanadium 31.0 g in ... weeks, an insignificant difference.

Survival rates. The percentage of animals surviving at each 3-month period are shown in figures 5–8. Increased mortality was exhibited by animals fed arsenic and germanium at one or more intervals compared with controls. Tin and vanadium did not appear to affect mortality at any age. The survival curves of males receiving vanadium and tin and their controls, however, tended to be fairly straight, whereas those of males on arsenic and germanium and their controls were sigmoid-shaped. Such straight curves have been reported for male animals deficient in chromium (12, 13).

Life-span and longevity of mice. Median life spans of male mice fed arsenic and germanium were less than that of their controls by 74 and 92 days, respectively (table 1). Similarly, females fed arsenic and germanium had shortened median life spans by 70 and 35 days, respectively. No such shortening of life span occurred in mice receiving tin or

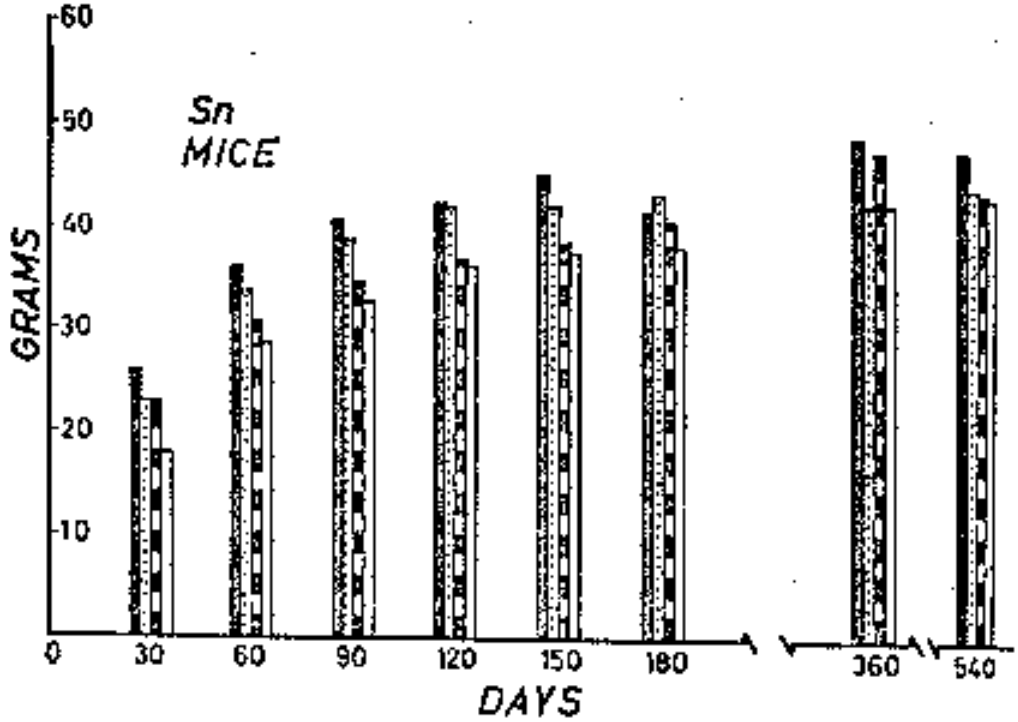

Fig. 3 Mean weights of mice receiving tin at various ages. The solid bars represent tin-fed mice, the dotted bars their controls. The hatched bars represent tin-fed females, the open bars their controls. There were no significant differences between controls and tin-fed animals at any age. The mice did not receive chromium; they were significantly lighter than the controls shown in figures 1 and 2 which had 1 μg/g in water, males differing by 2.8 to 9.02 g at various ages ($P < 0.05$–0.01) and females differing by 2.0 to 7.7 g ($P < 0.025$–0.0005).

vanadium. Male controls had a 60-day and females a 54-day longer median life-span when given chromium than when not; similar data have been reported previously (12).

Longevity, defined as the mean age at death of the oldest 10% of animals, was significantly less in male than in female mice given arsenic and germanium, and greater in males given tin (table 2). Compared with controls, mice of both sexes fed arsenic and males fed germanium had a lessened longevity.

Accumulation of metals. In all cases, the trace metals accumulated in one or more organs of mice, in some cases in sizeable quantities (tables 3–6). Accumulation with age was demonstrated for tin and germanium (table 7); the number of samples analyzed for vanadium and arsenic were too few to estimate age-linked accumulation; although the highest values for arsenic in spleen were noted in older mice (10 μg/g).

Incidence of spontaneous tumors. Significantly fewer spontaneous tumors were observed in mice fed arsenic, tin, in the controls which had 55, or those germanium which had 25, tin which had 22 or vanadium which had 15 ($P < 0.05$). These data are reported in detail elsewhere (14).

Other pathological changes. Examination of the microscopic sections revealed no consistent pathological changes associated with the ingestion of any of the four trace elements.

Discussion

These experiments, which are confined to mice, suggest no inherent or recognized toxicity of tetravalent vanadium in the form and dose given, in terms of growth, survival, longevity, life span or pathol-

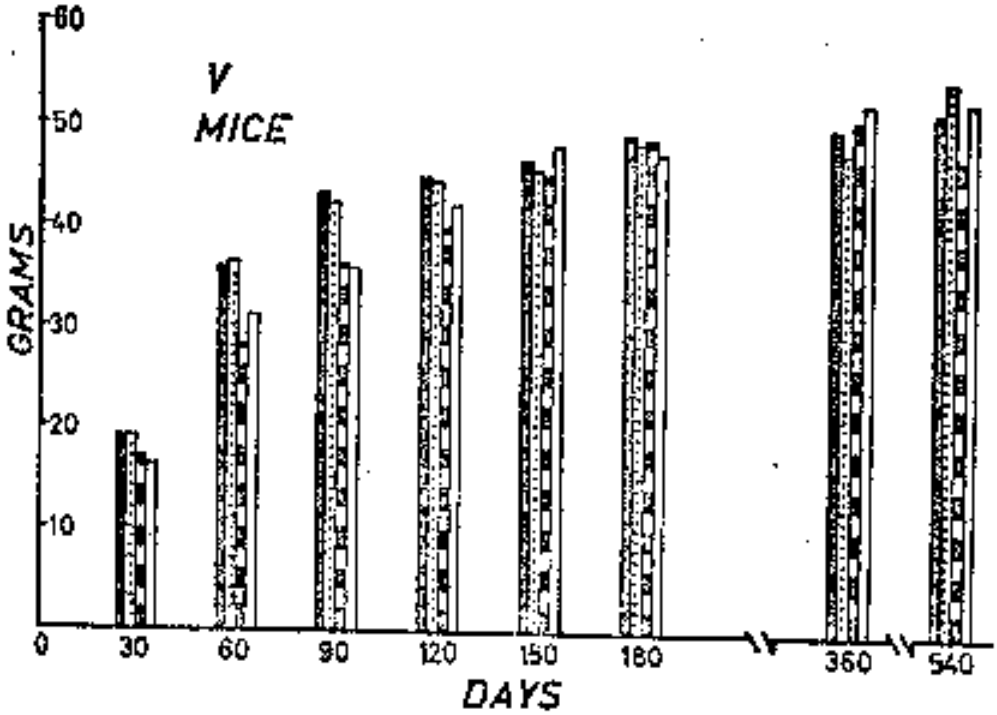

Fig. 4 Mean weights of mice receiving vanadium as vanadyl ion at various ages. Solid bars represent vanadium-fed males, dotted bars their controls; hatched bars females fed vanadium, open bars their controls. No significant differences appeared at any age. For the first 180 days these mice were fed a diet containing fish flour (see text), thereafter the regular diet. Chromium was not added to their water.

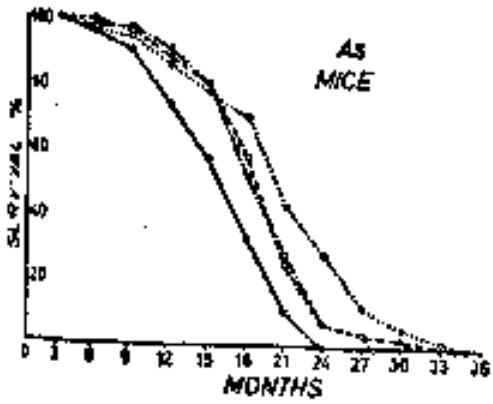

Fig. 5 Survival of mice given arsenic compared with that of controls. Solid lines show arsenic-fed males, dashed lines their controls; dot-dashed lines show arsenic-fed females, dotted lines their controls. By chi-square analysis there is a significant difference at 18 months in males ($P < 0.025$) and at 21 months in females ($P < 0.01$).

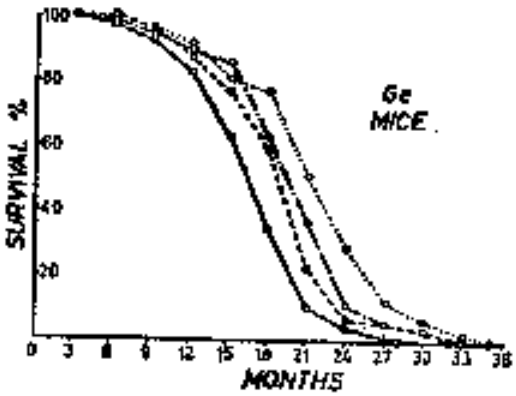

Fig. 6 Survival of mice given germanium compared with that of controls. Solid lines show germanium-fed males, dashed lines their controls; dot-dashed lines show germanium-fed females, dotted lines their controls. By chi-square analysis there is a significant difference at 18 months in males ($P < 0.005$) but not in females.

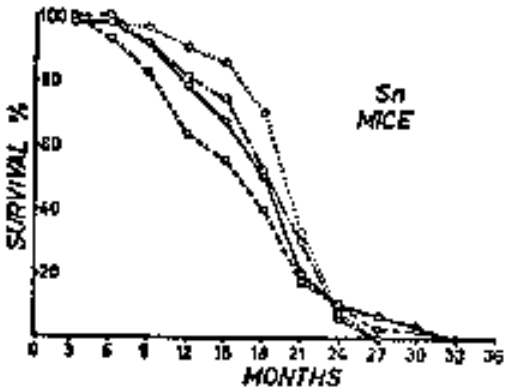

Fig. 7 Survival of mice given tin. Solid lines show tin-fed males, dashed lines their controls; dot-dashed lines show tin-fed females, dotted lines their controls. Male survival curves are relatively straight, characteristic of partial chromium deficiency. No significant difference in mortality was found at any age.

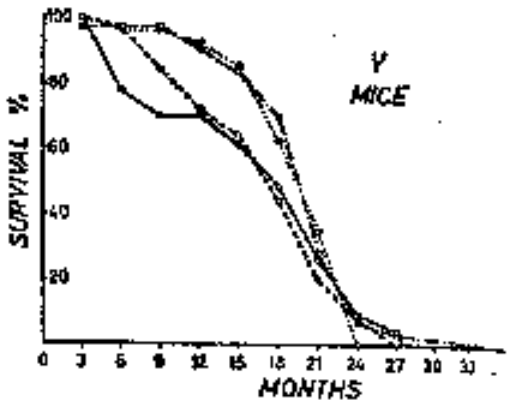

Fig. 8 Survival of mice given vanadium. Solid line represents male, dashed and dotted lines male controls; dashed line represents male, dotted line female mice fed vanadium. Male survival curves are relatively straight, characteristic of partial chromium deficiency. No significant difference in mortality in each sex was found at any age.

TABLE 1

Median life spans of mice given trace elements

	Males		Females		Difference
	No. animals		No. animals		
		days		days	days
Control [1,2]	39	510	72	570	60
Vanadium [1]	29	500	29	580	80
Tin [1]	54	548	54	554	6
Control [3]	54	570	54	624	54
Arsenic [3]	54	496	54	548	52
Germanium [3]	71	478	65	589	111
Total	315		328		

[1] No chromium added to drinking water.
[2] As no significant differences appeared in the median life spans of the controls for the vanadium-fed and those for the tin-fed animals, both control groups were combined.
[3] Chromium added to drinking water, 1 μg/ml.

TABLE 2

Longevity [1] of mice given trace elements

	Male		P value [2]	Female	
	Mean age	Maximal age		Mean age	Maximal age
	days	days		days	days
Control [3]	802 ± 50.2 [4]	1007	ns [5]	735 ± 29.0	789
Vanadium	779 ± 37.6	836	ns	803 ± 93.0	1084
Tin	896 ± 35.8	990	< 0.005	761 ± 14.1	806
Control [6]	831 ± 49.6	1016	ns	810 ± 45.0 [7]	1050
Arsenic	691 ± 7.4 [8]	700	< 0.005	789 ± 22.4 [9]	839
Germanium	712 ± 29.2 [8]	835	< 0.025	829 ± 35.9	971

[1] Longevity is the mean age of the last surviving 10% of animals in each group.
[2] Significance of difference between male and female values.
[3] No chromium added.
[4] Mean ± SE of mean.
[5] Not significant.
[6] Chromium, 1 μg/ml. added.
[7] Differs from first control value, $P < 0.005$.
[8] Differs from chromium control value, $P < 0.025$.
[9] Differs from chromium control value, $P < 0.05$.

TABLE 3

Arsenic in mouse tissues, wet weight[1]

	Controls		Arsenic-fed	
	No. animals		No. animals	
		μg/g		μg/g
Kidney	2	1.30	16	1.31
Liver	29	0.02	11	0.43
Heart	11	< 0.02	13	1.41
Lung	11	0.11	11	1.35
Spleen	2	5.60	15	3.03
Tumor	1	0.93	1	0.19

[1] Tissues were pooled in lots of 2–11. Means of both sexes included. Food contained 0.40 μg/g arsenic. The wild mice had no detectable arsenic in kidney, 0.02 μg/g in liver and 1.2 μg/g in heart.

TABLE 4

Germanium in mouse tissues, wet weight[1]

	Controls		Germanium-fed	
	No. animals		No. animals	
		μg/g		μg/g
Kidney	11	0.12	63	3.12
Liver	3	0.49	63	2.03
Heart	2	1.62	48	1.52
Lung	2	0.86	69	3.85
Spleen	11	0.28	68	11.37
Tumor			3	2.80

[1] Means of both sexes included. Organs were pooled in lots of 2–8. Food contained 0.32 μg/g germanium. The wild mice had 0.36 μg/g in liver and 2.49 μg/g in spleen.

TABLE 5

Tin in mouse tissues, wet weight[1,2]

	Males		Females	
	No. animals		No. animals	
		μg/g		μg/g
Kidney	29	1.70	37	3.32
Liver	29	1.24	37	2.26
Heart	43	2.95	32	5.04
Lung	32	4.47	37	1.60
Spleen	43	1.81	37	4.47
Fat			9	0.81
Thyroid	14	2.15		
Tumor			1	5.12

[1] Organs were pooled in lots of 2–14. Food contained 0.29 μg/g tin. Mean values shown.
[2] Too few control females were available for tabulation; they contained less than 0.5 μg/g tin.

TABLE 6

Vanadium in mouse tissues, wet weight[1,2]

	Controls		Vanadium-fed	
	No. animals		No. animals	
		μg/g		μg/g
Kidney			4	5.0
Liver	6	3.38	19	2.02
Heart	18	5.62	5	25.2
Lung	24	4.80	9	9.17
Spleen	17	7.38	11	19.86
Thyroid			5	17.60
Tumor	1	1.54	1	1.90

[1] Means of both sexes included. Organs were pooled in lots of 5–13. Food contained 1.4 μg/g vanadium for first 6 months of age, thereafter 3.2 μg/g (see text).

TABLE 7

Accumulation of tin and germanium in mice with age, organs of highest concentrations, wet weight[1]

Age	Tin			Germanium	
	No. animals	Heart	Spleen	No. animals	Spleen
days		μg/g	μg/g		μg/g
< 300				4	0.42
301–400	16	3.76	0.91	16	3.23
401–500	5	4.02	2.92	33	9.68
501–600	15	3.66	3.29	27	9.17
601–700	24	5.31	2.53	8	28.68
701+	3	5.14	24.39		

[1] Mean values of concentrations in organs of all animals dying in the intervals shown are included. Organs were pooled in lots of 2–14.

...al changes, despite accumulations in heart and spleen. Because of the relatively high concentrations of vanadium in the diets (vanadium is found concentrated in all biogenic lipids studied (4)), we were unable to demonstrate physiological effects by comparing growth and survival of animals fed little or no metal with those of normal amounts.

Although stannous tin accumulated in spleen, and, to less extent, in heart, only one effect of this element appeared. The median life span of males and females was almost identical, and longevity of males was greater than that of females. In our other series of mice, females usually lived longer than males (12). Therefore, innate toxicity of tin according to the

criteria studied was not demonstrable, despite probable accumulations.

Trivalent arsenic showed a slight tendency to accumulate in heart and lung of mice and, at the levels given, was slightly toxic in terms of median life span, longevity and survival, but not in terms of growth.

Although inorganic trivalent arsenic has been traditionally reputed to cause cancer in man, attempts to induce cancer with this element in mice and rats have consistently failed (15). The genesis and authenticity of the belief has been questioned (16). We observed fewer spontaneous tumors in mice given it at this level. The suppression of cancer incidence was apparently not associated with the decreased life span (14). Life-term experiments in rats, however, indicated no inherent toxicity of arsenic. These data will be reported.

Germanium accumulated in the spleens, kidneys, livers and lungs of mice, and at the level given, was slightly toxic in terms of life span, survival and longevity of males, but not of growth. Such subtle toxicity as appeared was confined to males. Germanium, however, has been said to show a low order of toxicity in acute experiments (16).

These experiments compared low levels of elements in the diet with higher levels in drinking water. A rough approximation of the comparable intakes can be made, assuming 7 ml of water * and 6 g food ingested per 100 g body weight per day (2). On this basis, the relative intakes of the controls and experimental animals per 100 g body weight per day, respectively, were: arsenic, 2.76 and 37.76 μg; germanium, 1.92 and 36.92 μg; vanadium, 8.4 to 19.2 and 43.4 to 54.2 μg; tin, 1.68 and 36.68 μg.

Because growth and survival of weanling and young mice were unaffected by any of these 4 elements, the levels given were tolerable. It is probable that longevity, late survival and life span of animals are more direct criteria of innate toxicity of elements than is the criterion of growth, especially at levels tolerated by the young.

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* It was almost impossible to obtain an accurate measurement of the intake of water by mice, due to their habit of stuffing food into the drinking tubes and causing losses from the bottles. The value represents the intake of mature rats weighed for a year.

Germanium, Tin and Arsenic in Rats: Effects on growth, survival, pathological lesions and life span [1]

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ABSTRACT To evaluate innate effects of the trace elements germanium, tin and arsenic, 455 rats of the Long-Evans strain were fed a diet containing small amounts of these elements in an environment relatively free of trace contaminants. Groups of 100 or more, divided as to sex, were given 5 ppm germanate, arsenite or stannous ions in drinking water from weaning until natural death and compared with an equal number of controls. These levels were tolerable for growth. Innate toxicity in terms of life span and longevity occurred in females given tin and both sexes given germanium; increased incidences of fatty degeneration of the liver were observed for both these elements. Animals fed tin also showed an increased incidence of vacuolar changes in their renal tubules. Large amounts of arsenic accumulated in tissues, especially aorta and red blood cells, with no signs of toxicity. Tin accumulated to some extent and germanium less so. No element was tumorigenic or carcinogenic, there being somewhat fewer tumors in rats fed germanium than in the controls.

Life-term studies on mice and rats exposed to several abnormal trace metals have been reported (1–4). A more recent study involved germanium, tin and arsenic in mice (5). To confirm and extend these observations, rats were maintained on similar regimens. One purpose of these studies is to discover, if possible, recondite toxic effects in terms of growth, survival and extreme life span; another is to discover which elements, given orally, accumulate in several organs with age. In mice tin was found to accumulate in heart muscle, as it does in man (6), and in spleen. Germanium accumulated in spleen, and arsenic given as arsenite to a small extent in liver, heart and lung. Mice fed germanium and arsenic had shorter survival times than their controls. The present report considers these and other studies in rats.

METHODS

In methods and materials used, the experimental diet, the drinking water and the general environmental conditions of the laboratory have been reported in detail and have not been altered (1). Random-bred rats of the Long-Evans strain were purchased and their offspring were born and reared in our laboratory. Groups of 50 or more of each sex, 4 in a cage, were given at weaning time sodium germanate, stannous chloride or sodium arsenite in drinking water at levels of 5 µg/ml of the element. The water also contained 1.0 µg/ml chromium (III) as the acetate, as well as soluble salts of zinc, copper, manganese, cobalt and molybdenum (1).

Animals were weighed at weekly intervals when weanlings, then at monthly intervals. They were disturbed for measurements of blood pressure, requiring anesthesia; for sampling of blood for analyses of fasting serum glucose levels and cholesterol, and for cleaning their cages at weighing time. At natural death, they were weighed, dissected and gross pathological changes described. Heart, lung, kidney, liver, spleen and tumors were fixed in Bouin's solution, sectioned, stained with hematoxylin and eosin and examined under light microscopy. Portions of these tissues were frozen in polyethylene bottles and at a suitable interval, ashed and analyzed for the elements given. A low tem-

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perature asher[4] was used for arsenic (7) and germanium (8), as these elements are volatile; in the case of tin (6), tissues were ashed at 480° in muffle furnaces. Analytical methods used and their sensitivities have been reported in the study on mice (5).

Serum glucose was measured by the glucose oxidase method of Washko and Rice (9), using premixed reagents and a Berkeley Medical Instrument spectrophotometer.[5] Duplicate and replicate analyses agreed within 4%. Blood samples for fasting levels were obtained from 12 rats of each sex and each group, after they had been deprived of food for 18 hours.

Urine was tested semiquantitatively by sensitized paper[6] for protein and glucose. Blood pressure was measured on warmed, anesthetized animals by the method of Friedman and Freed (10).

During the summer of 1963, several studies involving general anesthesia and blood letting were made on female control animals, resulting in excessive mortality between the ages of 24 to 27 months. This mortality was confined to 5 cages. To provide adequate controls, the data on survival of the remaining 8 cages (32 animals) were combined with those of 11 cages (44 animals) in the control series previously reported (4). Erroneous comparisons would have resulted if the whole series of female controls were used. Survival of the 32 female rats used was similar to that of the 44 animals added. Growth rates were measured on the original series of 52 animals.

The lesions found in microscopic sections were evaluated by methods reported previously (11). Lesions were graded zero to 3+. Two changes in the liver were recorded: fatty degeneration, and cellular necrosis of peripheral, medial and central portions of the lobule. One lesion in the kidney was recorded: vacuolar changes in the renal tubules. The presence of pyelonephritis and other disorders was noted but not evaluated in this study, as no differences were found between groups. Blood vessels were examined for changes in their walls in heart, kidney, liver, lung and spleen.

The diet of seed rye flour (60%), dry skim milk (30%), corn oil (9%) and sodium chloride (1%), to which vitamins and ferrous sulfate were added (1), contained the following amounts of the trace elements under study (in μg/g wet weight): germanium 0.32, arsenic 0.46 and tin 0.28.

RESULTS

Growth rates. None of the 3 elements affected growth rates of rats significantly except at isolated intervals (table 1). At 18 months of age, however, males fed arsenic and germanium weighed less, and females fed germanium weighed more than their controls. Otherwise, mean weights of the 3 groups were remarkably similar. In the first few months the male rats in the series, given 5 ppm chromium in water, grew somewhat more rapidly than those of the series reported previously (2), but by month 6 their weights were similar and so remained. Female rats of this series were larger at all ages including 1 year.

Heart weight. The mean body weight at death, the mean weight of the heart and their mean ratios are shown in table 2. Hearts of male rats fed arsenic were smaller than those of their controls. Differences among other groups did not appear.

Survival. Median, 75% and 90% life spans are given in table 3 and the survival curves in figures 1 and 2. Differences of 2 months or more occurred in 3 groups at three intervals. Rats of both sexes fed arsenic and males fed tin were unaffected as to survival, according to these data, whereas both sexes given germanium and females given tin lived somewhat less long at these intervals. The difference of 1[illegible] days in the median life span of males fed germanium was significant.

Longevity. Defined as the mean age of the last surviving 10% of animals in each group, longevity is shown in table 3. Significantly lessened longevity was found in female rats given tin, and it was less by more than 2 months in females fed germanium. In the table also are the ages of the oldest survivors. A male fed arsenic lived a year longer than any of the others.

[4] Tracerlab, 500 A. Richmond, California.

[5] Berkeley Medical Instruments, New England X-Ray and Electronic Equipment, Brookline, Massachusetts 02146.

[6] Combistex, Ames Company, Elkhart, Indiana.

TABLE 1

Mean weights[1] of rats given arsenic, germanium and tin

Age	Control	Arsenic	Germanium	Tin
days	g	g	g	g
Males				
30	72 ± 4.2	65 ± 4.1	79 ± 5.8	87 ± 6.3 [2]
60	190 ± 6.0	186 ± 8.8	193 ± 11.2	181 ± 8.2
90	270 ± 8.0	211 ± 6.7 [3]	272 ± 11.3	261 ± 7.7
120	313 ± 9.3	307 ± 4.9	305 ± 8.7	297 ± 7.7
150	342 ± 8.0	335 ± 5.2	339 ± 8.5	336 ± 8.6
180	365 ± 8.7	350 ± 7.2	363 ± 8.6	355 ± 11.3
360	444 ± 14.9	431 ± 6.5	464 ± 14.7	447 ± 10.4
540	507 ± 16.1	479 ± 11.0	453 ± 8.7 [4]	463 ± 10.5 [3]
Females				
30	65 ± 2.1	73 ± 4.8	64 ± 1.9	87 ± 4.9 [4]
60	154 ± 6.0	148 ± 7.4	132 ± 5.1	149 ± 6.7
90	197 ± 5.4	190 ± 4.0	183 ± 4.6	189 ± 5.5
120	225 ± 5.3	206 ± 5.5	213 ± 4.8	213 ± 4.0
150	239 ± 4.1	235 ± 4.9	235 ± 4.5	232 ± 3.6
180	251 ± 4.0	212 ± 5.0	243 ± 4.5	241 ± 3.8
360	268 ± 5.9	265 ± 6.5	286 ± 6.3 [4]	279 ± 7.0
540	287 ± 9.8	274 ± 5.4	301 ± 9.2 [4]	281 ± 5.2

[1] ± = SEM. There were 52–58 rats in each group.
[2] Differs from control, $P < 0.05$.
[3] $P < 0.025$.
[4] $P < 0.01$.
[5] $P < 0.005$.

TABLE 2

Heart and body weights of rats given arsenic, germanium and tin

	No. rats	Heart wt[1]	Body wt[1]	Ratio × 1000
		mg	g	
Males				
Control	49	1498.1	333.9	4.88 ± 0.206
Arsenic	51	1340.1	332.1	4.02 ± 0.188 [2]
Germanium	49	1107.8	316.8	4.65 ± 0.186
Tin	44	1442.0	297.3	5.07 ± 0.243
Females				
Control	41	948.5	234.0	4.45 ± 0.216
Arsenic	44	1061.9	230.7	4.93 ± 0.231
Germanium	44	987.2	228.3	4.51 ± 0.211
Tin	51	914.8	197.8	4.65 ± 0.197

[1] At death.
[2] Differs from controls, $P < 0.025$.

TABLE 3

Mean life spans of rats given arsenic, germanium and tin

	No. rats	50% Dead	75% Dead	90% Dead	Longevity[1]	Oldest
		days	days	days	days	days
Males						
Control	58	872	974	1057	1100 ± 27.8	1232
Arsenic	53	825	937	1093	1240 ± 96.0 (1120 ± 18.4) [2]	1506
Germanium	31	738 [3]	902	1017	1177 ± 58.8	1319
Tin	56	876	957	1097	1134 ± 22.8	1222
Females						
Control	78	912	1050	1157	1304 ± 36.0	1347
Arsenic	55	912	1056	1175	1249 ± 21.9	1315
Germanium	52	833	932	1057	1231 ± 25.0	1308
Tin	55	830	989	1094	1160 ± 27.5 [3]	1207

[1] See text. Mean ± SEM.
[2] Excluding last survivor, which lived 376 days longer than next to last survivor.
[3] Differs from controls, $P < 0.005$.

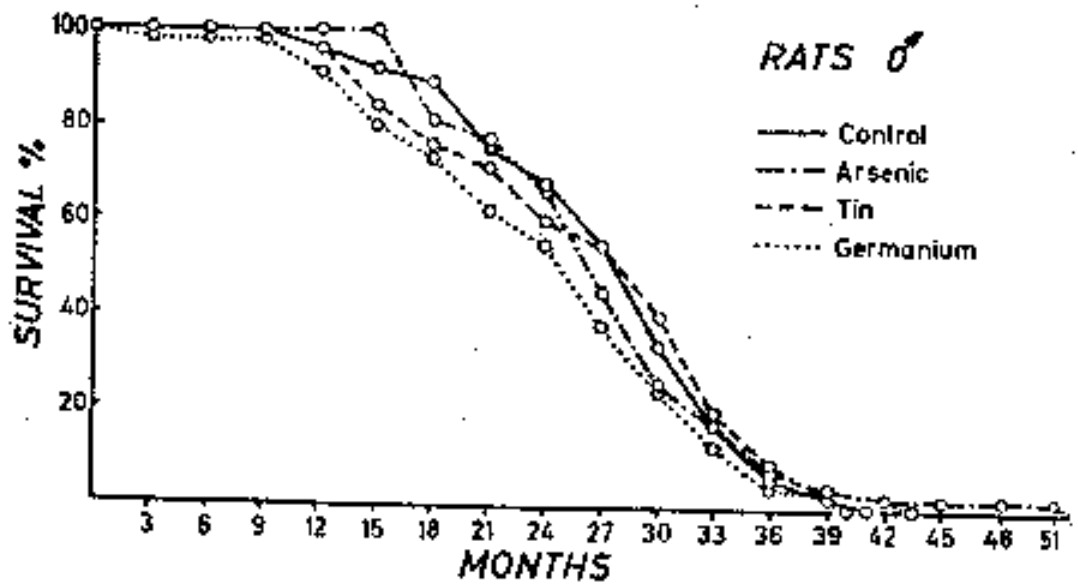

Fig. 1 Survival curves of male rats fed arsenite, germanate and stannous ions (5 ppm) in drinking water from the time of weaning. Although there were no significant differences from the controls at any 3-month interval, animals fed germanium survived somewhat less well from 9 to 27 months of age. Note the long survival of one animal fed arsenite.

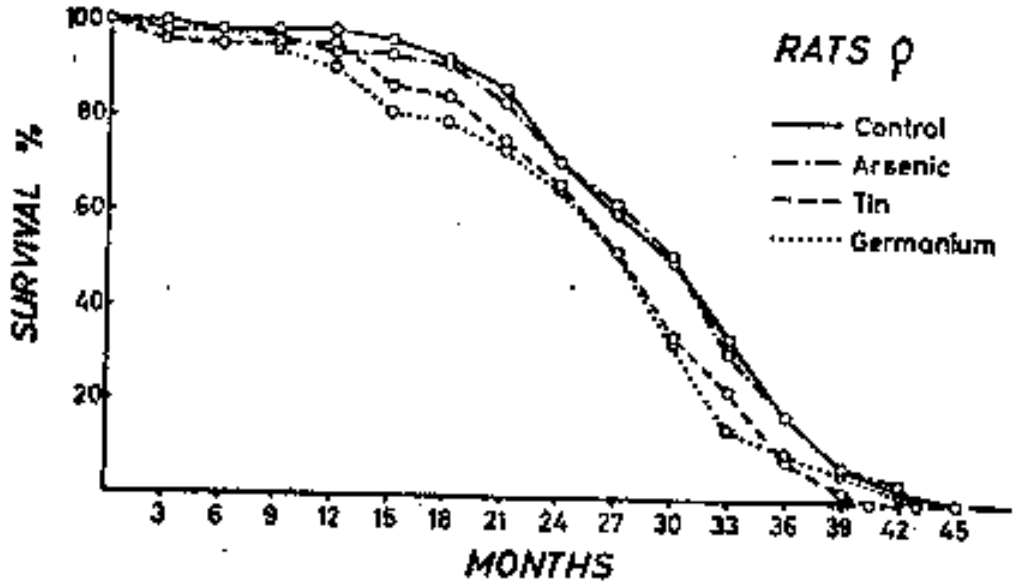

Fig. 2 Survival curves of female rats fed arsenite, germanate and stannous ions (5 ppm) drinking water from the time of weaning. Although no significant differences from the controls were found for the rats fed tin, their survival was slightly less from 15 to 33 months of age. Animals fed germanium differed from the controls at 33 months of age by chi-square analysis ($P < 0.0$ and, in general, survived less well than their controls.

controls, more than 52 months, which was 111 days longer than our other oldest animal, one fed chromium (12).

Blood and urinary findings. Serum cholesterol levels on these rats have been reported (13); they were similar in all female groups. Males given arsenic had higher mean values ($P < 0.05$) and males given germanium had lower mean values ($P < 0.001$) than their controls.

Mean fasting serum glucose levels in male rats (± SEM) and the significance of differences in the values of the three groups from the controls were (in mg/100 ml): controls, 106.5±3.6, arsenic 78.5±4.4 ($P < 0.001$); germanium, 108.0±4.7; and tin, 101.3±4.2. In female rats they were: controls, 79.6±8.2; arsenic, 77.8±5.8; germanium, 99.7±3.6 ($P < 0.01$); and tin, 100.0±4.8. ($P < 0.001$). These measurements were made at approximately 2 years of age for the controls, 26 months for the arsenic group, 19 months for the germanium group and 16 months for the tin group.

Glycosuria was found in 15 of 66 control rats, 9 of 20 in the arsenic group, 3 of 21 in the germanium group and 6 of 22 in the tin group. It was of slight degree in all cases but one control, in which it was marked. Differences between groups were not statistically significant.

Proteinuria was common to almost all rats. Severe grades (3+ and 4+) were found in 27.3% of 66 control animals, [illegible]% of those in the arsenic group, [illegible]% of those in the germanium group and 36.4% of those in the tin group. For these two grades differences between groups were not significant. Grades 2 and [illegible], however, occurred in 90% of the rats fed germanium and 63.7% of the controls ($P < 0.05$).

Blood pressure. Ten mature male and [illegible] female animals from each group were randomly selected for measurements of blood pressure at approximately 2 years of age. No abnormalities were found in three consecutive measurements at monthly intervals.

Tumors. We have reported that mice fed germanium had fewer tumors in all sites and those fed arsenic had fewer tumors of the lung than did their controls. Herein the feeding of tin was associated with no such effect (11). In rats, a difference appeared only in the germanium group. Tumor incidences in the 4 groups were as follows: controls 37.8%, arsenic 27.8%, germanium 22.2% ($P < 0.05$) and tin 29.9%. Clusters of abnormal liver cells appearing "preneoplastic" (11) were more frequent in the arsenic group (20%) than in the others (4-10%). Of the 18 malignant tumors found, there were 9 in the controls, 3 in the arsenic group, 2 in the germanium group ($P < 0.05$) and 4 in the tin group. These data will be reported elsewhere.

Other pathological changes. The degrees of fatty degeneration of the liver found in the various groups are shown in table 4. Included are livers from previously reported animals (4) fed chromium and cadmium. There was a high incidence of fatty change in the livers of the germanium- and tin-fed rats compared with the controls, and a low incidence in the chromium group. Hepatic cellular necrosis occurred in all groups (12.1 to 22.2%) (table 5). Vacuolar changes in the proximal convoluted tubules of the kidney were significantly increased in animals fed tin (36.2%, $P < 0.025$) compared with controls (18.2%) (table 4). Vascular lesions were singularly rare or absent in the various groups.

Accumulation of elements in tissues. Mean values of the elements in tissues are shown in tables 6 through 8. Compared with controls, these elements accumulated in one or more organs.

Nine analyses on 23 stillborn or suckling rats showed no detectable tin in their whole carcasses. Three contained 0.68 μg/g. Certain tumors accumulated tin in concentrations of 0.91 to 7.77 μg/g, 5 containing more than 2.73 μg/g, whereas 4 other tumors had no detectable tin. Hearts and spleens showed more tin than did other normal tissues (table 6), which was also the case with mice (5). Accumulations were not large and, although the differences from the controls were not statistically significant, the prevalences were, in kidney, liver and lung.

Arsenic accumulated with age in all tissues analyzed to a remarkable extent. Although few young rats were available

TABLE 4

Fatty degeneration of the liver and vacuolar changes in the proximal convoluted tubule of the kidney in rats given trace elements [1]

	No. rats	Severity of lesion			Total no.	With lesions
		+	++	+++		%
Liver						
Control	88	6	22	5	33	37.5
Arsenic	83	12	26	4	42	50.6
Germanium	86	15	32	7	54 [3]	62.8
Tin	80	17	30	7	54 [4]	67.5
Chromium [2]	29	1	4	0	5 [4]	17.9
Cadmium [2]	24	2	4	2	8	33.3
Kidney						
Control	88	9	7	0	16	18.2
Arsenic	77	14	8	3	23	29.9
Germanium	84	14	7	1	22	26.2
Tin	81	15	10	1	26 [3]	32.1
Chromium [2]	27	2	1	0	3	11.1
Cadmium [2]	33	4	0	0	4	11.4

[1] Fatty changes in the liver were classified as peripheral, central and diffuse. There appeared to be no significant difference in the [illegible] among the various groups, except in rats fed chromium where peripheral fatty changes were [illegible].
[illegible]
[illegible]
[illegible]

TABLE 5

[illegible]

	No. rats	[illegible]	[illegible]	Total	%
[illegible]	58	[illegible]	[illegible]	44	[illegible]
Arsenic	41	[illegible]	[illegible]	63	[illegible]
Germanium	56	[illegible]	[illegible]	[illegible]	[illegible]
Tin	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Chromium [2]	25	[illegible]	4	[illegible]	[illegible]
Cadmium [2]	24	9	6	15	62.5

[1] Included from table 4. Differs from controls by chi-square analysis, $P < 0.05$.
[2] Included from previous studies (4) for comparison.
[3] Number differs by chi square analysis, $P < 0.05$.

TABLE 6

Tin in tissues of rats, wet weight

	Controls [1]			Fed tin [2]		
	No. rats	No. present	Mean	No. rats	No. present	Mean
			μg/g			μg/g
Kidney	21	7 [3]	0.31	33	33	0.17
Liver	21	16 [4]	0.11	32	33	0.35
Heart	21	18	0.59	32	32	0.93
Lung	15	7 [3]	0.14	32	32	0.54
Spleen	21	21	0.98	36	36	1.88
Stillborn, whole	26	3	0.09	—	—	—
Tumors	18	11	1.77	2	2	1.12

[1] Control rats were 533 to 822 days old.
[2] Tinfed rats were 802 to 933 days old.
Tissues were pooled in groups of 3 to 11 samples.
Differences between the means of the control and rats fed tin were not statistically significant.
[3] Differs from group fed tin by chi-square analysis, $P < 0.001$.
[4] $P < 0.01$.

for analysis because of the low mortality of this group, 10 aged less than 2 years had (in μg/g): kidney 9.20, liver 5.83, heart 11.90, lung 10.89, and spleen 14.18. Older rats had several times these values (table 7). Red blood cells of aged rats took up as much as 282 μg/g arsenic. This element also accumulated in the aorta. The oldest animal fed arsenic had lower concentrations in five tissues than the means of the others. Small amounts were found in most tissues of control animals and differences were highly significant. No arsenic was detected in kidneys, or in 16 lungs. This large accumulation of arsenic in rats can be compared with the relatively small amounts found in mice treated identically (5).

Germanium accumulated in the kidney (table 8) and was present in more livers and lungs than in controls; however, relatively small amounts were found.

TABLE 7

Arsenic in rat tissues, wet weight [1]

	Control [2]		Fed arsenic		
	No. rats	Mean	No. rats	Mean	Range [3]
		μg/g		μg/g	μg/g
Kidney	27	0.0	59	37.63	11–49
Liver	39	0.21	91	46.92	23–169
Heart	11	0.53	90	31.53	10–87
Lung	11	0.25	96	46.19	22–123
Spleen	11	0.31	83	39.79	14–99
Brain	3	0.38	—	—	—
Tumor	3	0.25	6	30.78	3–75
Aorta, F (dry wt) [4]	—	—	10	316.60	—
Aorta, V (dry wt) [5]	—	—	10	108.88	—
Washed RBC					
Young [6]	—	—	12	73.90	—
Old [7]	—	—	24	282.46	240–316
Whole blood	—	—	1	44.79	—

[1] Tissues were pooled in lots of 11 to 10.
[2] Control rats were 583 to 938 days old.
[3] Rats more than 2 years old.
[4] Less than 2 years old.
[5] More than 2 years old.
[6] Less than 1 year old.
Note: Oldest rat aged 1396 days had (μg/g): muscle 5.71, skin 2.23, hair 92.40, teeth 1.09, femur 4.04, kidneys 24.74, heart 19.05, lung 60.11, spleen 11.49, liver 20.75. Differences in the means of control and rats fed arsenic were highly significant ($P < 0.001$).

TABLE 8

Germanium in the tissues of rats, wet weight [1]

	Controls [2]			Fed germanium [3]		
	No. rats	No. present	Mean	No. rats	No. present	Mean
			μg/g			μg/g
Kidney	25	25	0.18	24	24	0.02 [4]
Liver	25	10 [5]	0.14	25	25	0.07
Heart	12	6	0.16	22	22	0.17
Lung	18	13 [6]	0.11	34	31	0.28
Spleen	9	9	0.58	18	18	0.50
Tumor	2	2	1.05	—	—	—
Whole, newborn	1	1	0.18	—	—	—
Fat	—	—	—	1	1	0.53

[1] Tissues were pooled in groups of 4 to 10.
[2] Control rats were 500 to 913 days old.
Germanium-fed were 510 to 893 days old.
[4] Means of pooled samples differ from controls. $P < 0.025$.
[5] Differ from group fed germanium by chi-square analysis, $P < 0.0001$.
[6] $P < 0.005$.

DISCUSSION

In these and previous studies we have attempted to evaluate several trace elements present in human food and in the environment for effects on growth and longevity and for recondite toxicity in terms of chronic disease. Two common disorders have appeared in our laboratory, hypertension caused by excess cadmium (14) and diabetes mellitus resulting from chromium deficiency (12). This study uncovers no suggestion that specific disorders may result from arsenic, germanium or tin. None of these elements was tumorigenic or carcinogenic, nor was any associated with overt disease. No arsenic keratoses appeared, and this element was not toxic in terms of growth and life span.

In attempting to evaluate less obvious changes in the various criteria used in this study, we can arbitrarily classify values different from the controls as favorable or adverse. Thus, limited growth, lessened survival and longevity, elevated serum cholesterol and glucose levels, excess proteinuria, excess number of tumors, fatty changes in the liver and renal lesions can be considered as adverse, whereas their counterparts can be considered as favorable. In this light, female rats fed arsenic showed no significant differences from the controls; males had elevated serum cholesterol levels only. Glucose levels, however, were lower than the controls. These very few changes occurred in the presence of a remarkable accumulation of arsenic in the tissues, demonstrating that arsenic, given at this level, is not toxic to rats.

In animals fed tin, males showed one adverse effect, lower body weight; females exhibited decreased survival and longevity and elevated glucose levels, and both had increased incidences of fatty changes in liver and renal tubular vacuolization. No criterion was altered in a favorable direction. These changes were found in the presence of only small amounts of tin in tissues.

In rats given germanium, changes considered adverse were greater. Excess proteinuria, fatty livers and decreased survival were found in both sexes; males had lower weights and females decreased longevity and moderate hyperglycemia. Concerning presumed favorable effects, bod weight in females was increased, serum cholesterol in males was decreased an there were fewer tumors. These chang occurred in the presence of slight accumu lations of germanium in tissues. This data suggest therefore that both germ nium and tin are slightly toxic to rats the levels fed. Germanium showed simil toxicity to mice (5), although we did n find fatty degeneration of the liver i creased in mice fed these elements.[7]

The approximate intakes of these el ments were (in μg/100 g body weigh day): arsenic 37.76, germanium 36.5 and tin 36.63, while the controls ingest 2.76, 1.92 and 1.68, respectively. These i takes are relatively much larger than the of human beings, who take in food a water about 900 μg arsenic (7), 1500 germanium (8) and 4.0 mg tin (6) or 1. 2.1 and 5.7 μg/100 g body weight, resp tively. Extreme but "normal" human i takes of arsenic and tin have been calc lated as 4.8 mg and 40 mg, respectiv (6.9 μg and 57 μg/100 g body weigh which places the intake of tin by r comparable to that of certain hum beings.

We have analyzed a few human tissu for arsenic. It was not detected in t kidney, liver, heart and pancreas of 9-month-old male, but was found (μg wet weight) in lung, 0.69; in muscle, 0.2 and in spleen, 1.43. In the tissues of a year-old female, arsenic was not deter in kidney, heart and spleen, but occur in liver (0.66 μg/g) and lung (0.59 μg/ These concentrations are similar to th found in control rats.

We have not analyzed human tissu for germanium. Concentrations of t found in rats fed this element were sligh larger in some organs than those repor in human beings (8), but were general similar. Therefore, the human experien with respect to tin was reasonably r duplicated in rats. At these tissue conc trations, tin exhibited recondite toxicity female rats in terms of life spans and s vival, fatty degeneration of the liver a vacuolar degeneration of the renal tubul This effect was not observed in mice (5

[7] Schroeder, H. A., M. Kanisawa, D. V. Frost, M. Mitchener, unpublished observations.

The levels of these elements given to rats were tolerable in terms of growth. The level of arsenic was tolerable in terms of survival despite large accumulations of this element. Byron et al. (15) found no effect on survival of rats given arsenate or arsenite at much higher levels than we used. The level of germanium, however, was associated with decreased survival. Such an effect was curious since little germanium accumulated in tissues. Apparently germanium as germanate is rapidly excreted via the urine (8), but despite this characteristic, it apparently can exert recondite toxicity on liver, kidney, and perhaps on growing tumor cells.

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Some remarks on the therapeutic activity of some tin derivatives in function of the histochemical detection of this metal (*)

by J. Turchini, L. Khau Van Kien, P. Castel, and Thai Tuong.

During our previous research, we had the opportunity of detecting histochemically some drugs containing tin, which have been introduced into the organism during intoxications and during therapeutic experiences on rats and rabbits. The results of the localizations of tin derivatives administered orally or injected subcutaneously, have brought us to deduct the toxic and therapeutic effects of these products in the treated animals.

Tin detection is based on techniques which are aimed at the conservation of tin derivatives due to the fixation of formol vapors and of different histologic fixers which curtail the diffusion and the loss of these products, and due to the use of three types of reactions in order to detect the metal under its various forms: mineral, organic, soluble or insoluble: 1° a dithiol reaction with pH 2 detects metallic tin by a red coloration, whereas organic or combined tin do not undergo this reaction; 2° sulphurized hydrogen in acetonic solution with pH 2 detects metallic and slightly combined tin; 3° hematein in acid or alkaline medium can result in a colored reaction with the different tin combinations: mineral tin becomes violaceous red in acid medium and violaceous in alkaline medium, organic tin is violaceous in

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(*) Report presented to the Societe de Therapeutique et de Pharmacodynamie, on April 27, 1958.

brown in alkaline medium, combined tin or tin masked by proteins becomes chocolate brown to dark brown. The reaction through formation of coloured hemateine-tin lakers was improved by the use of a penetrating and apolar vector, and it is carried out on congelation sections, or on whole pieces, or in particular cases on paraffine sections.

During our intoxication experiences and therapeutic experimentations we observed the effect of some tin containing drugs such as tin oxyde, stannous ~~tin~~ chloride or the association of both, dissolved stanoxyl, tin dibutyl-malleate and tin diiododiethyl. These products were administered orally, subcutaneously, by intraperitoneal or intravenous way in rats and rabbits, normal as well as parasite stricken, during a period which varied from several days to six months. The tin drugs were followed in the organism by their histochemical localization and their histopathological effects on tissues and organs. The results of these histochemical localizations, as well as the observation of sections and animals during treatments, have enabled us to appraise the toxicity of these products, their possible therapeutic effect in relation to their nature (insoluble, soluble, mineral, organic), their ionisation degree (more or less resorbable) and their means of administration.

An investigation of the results at the level of the points of introduction, of the ways of transit, of fixation and elimination points, can provide some very useful pieces of information.

We have primarily examined tissues and organs where tin derivatives have had a therapeutic and antiparasitic intra- and extra-intestinal part, particularly at the level of the digestive tract, the liver, the spleen, the kidneys, the lungs and vessels, their anti-microbic effect at the level of the skin and hair, nails and teeth, and event-

ually we have given their remote toxic effect at the level of the brain.

Within the digestive tract, at the level of stomach and intestine, we have examined the effect of tin oxyde and tin chloride in powder form, either alone or associated, added to food. When they are administered orally, the tin derivatives tend to pass quite rapidly through these organs: tin is found at the level of the mucous membrane of the stomach, within the folds and/lights of glands and particularly at the level of the mucous membrane of the intestine. These mucous membranes are heavily colored by the reagents, thus showing a massive localization. The product appears to pass through the stomach without being resorbed, whereas resorption is is higher in the intestine, but cytologic observation is very difficult. It should be noted that the mucous membrane is heavily affected by the causticity of those derivatives of tin. In cases of intoxication, stomach and intestine lose their tonicity and elasticity, tear very easily and display numerous ulcerations. Mineral and organic tin, when it is slightly ionized, can cross the intestinal barrier and penetrate into the intestinal vessels, the vessels of the liver, into the spleen, the kidneys, the lungs, etc. The degree of absorption varies with the nature of the absorbed product. Metallic tin is little harmful, insoluble mineral tin (oxyde and chloride) which is ionizable, crosses the intestinal barrier, and so do the mineral and organic soluble derivatives which go still further as they can be traced in the nervous system, in the skin, and various emunctories. We could not trace tin in tissues and mucous membranes of stomach and intestine if it was administered parenterally. Antiparasitic intra-intestinal activity is linked to the causticity of tin salts, and to the effect of parasited and their enzymes on proteins. Tin is

to be slightly ionized and not very soluble if it is to have an efficient action on intestinal parasites; mineral salts would be best with an identical quantity and a lower toxicity. Tin dibuylmalleate, which is organic and insoluble, is active if administered orally. As it is more resorbable, it presents extra-intestinal actions.

In the liver of rats and rabbits, tin can be found in substantial quantities, and its presence can be invoked for an explanation of activity on intra-hepatic parasites. Mineral derivatives which are not very soluble (anhydrous oxyde or hydrated oxyde) or organic (dibutylmalleate) are resorbed by the mucous membrane at reasonable doses and go into the liver. At small doses and with a reasonable duration, they can carry out a hepatic impregnation which is superior to that obtained with soluble mineral and organic derivatives administered parenterally. If they are administered at heavy doses they can penetrate into the liver only if the defense system is outflanked; at small doses, on the other hand, and if they are administered slowly, they can penetrate into the hepatic parenchyma and carry out some activity. These results of hepatic localizations could explain the successes observed in the treatment of bilharzioses and rots.

In the lungs of the same animals, injected mineral soluble and insoluble derivatives can penetrate into the pulmonary parenchyma where they are fixed and be eliminated by nucleated cells and free cells of the pulmonary cavity. Organic derivatives such as tin diiododiethyl, that are more soluble and more toxic, penetrate less through the same way, however, they can be found in more or less ~~congested~~ congested vessels; if they are conveyed in this way, they could go farther and penetrate into the nervous system. In our animals,

orally administered mineral derivatives can be traced back only later in the lungs, and during a longer trestment. The oresence of tin in the parenchyma can be a clue towards the explanation of the therapeutic power of some tin derivatives in the treatment of influenza and pulmonary affections, and tuberculosis in particular.

Orally or subcutaneously administered tin derivatives are also found in the skin, nails, hairs and teeth. They can be observed in the subcutaneous tissue, in the muscles, in the vessels and particularly in the hair follicles. Tin is eliminated by skin and hairs, as it can indeed be found in the root, in the internal epithelial sheath and in the cells of the marrow of the hair, according to the duration of treatment. Its presence within the skin and above all at the level of the hair follicles could explain the therapeutic activity of tin salts in the treatment of furunculosis and of staphylococcus anthrax.

Mineral derivatives can be found in the brain in the form of traces in the vessels of the pia mater; parenteral administration results in a slighter localization than oral administration. Organic tin, such as tin diiodoethyl, in intravenous injection, can be found in the vessels of the pia mater, in intra-cerebral vessels, and at the level of the white and grey matter. With heavy doses in the medial region of the gray cortex, near vessels, some pyramidal more or less altered cells can be found that show diffuse traces of tin in their protoplasma. Although tin is only slightly combined with proteins, certain signs of nervous intoxication and of alteration of nerve cells can be noted. It can be assumed that diiodoethyl and organic salts of this type are toxic and thus one can explain the observed therapeutic

accidents. The solubility of these organic tin products in lipids, as we have seen with GRANEL in the lung cells, can also contribute to a certain extent towards the explanation of neurotopic action of these substances on the brain, which is rich in lipids.

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Remarques sur l'activité thérapeutique de quelques dérivés de l'étain à la lumière de la détection histochimique de ce métal (*)

Par J. TUBCHINI (**), L. KHAU VAN KIEN, P. CASTEL et THAI TUONG

Au cours de nos recherches antérieures, nous avons eu l'occasion de détecter histochimiquement des médicaments stanniés, introduits dans l'organisme au cours d'intoxications ainsi qu'au cours d'essais thérapeutiques chez le Rat et le Lapin. Les résultats des localisations des dérivés stanniés administrés *per os* ou injectés sous la peau nous ont permis de déduire les effets toxiques et thérapeutiques de ces produits chez les animaux traités.

La détection de l'étain est basée sur des techniques visant à la conservation des dérivés stanniés grâce à la fixation aux vapeurs de formol et de divers fixateurs histologiques évitant la diffusion et la perte des produits, et à l'emploi de trois types de réactions pour détecter le métal sous ses différentes formes : minérale, organique, soluble ou insoluble : 1° la réaction au dithiol à *pH* = 2 détecte l'étain métallique par une coloration rouge, l'étain organique ou combiné échappe à cette réaction ; 2° l'hydrogène sulfuré en solution acétonique à *pH* = 2 détecte l'étain métallique et l'étain faiblement combiné ; 3° l'hématéine en milieu acide ou alcalin peut donner avec les différents composés stanniés des réactions colorées : l'étain minéral est coloré en rouge violacé en milieu acide et violacé en milieu alcalin, l'étain organique est violacé en milieu acide et brun violacé en milieu alcalin, l'étain combiné ou masqué par des protéines se colore en brun chocolat à brun foncé. La réaction par formation de laques hématéine-étain colorées a été améliorée par l'emploi d'un véhicule pénétrant et opalin, elle s'effectue soit sur des coupes à congélation, soit sur des pièces entières, soit encore sur des coupes à la paraffine dans des cas particuliers.

Au cours de nos expériences d'intoxications et d'essais thérapeutiques nous avons pu observer l'effet de quelques médicaments stanniés comme l'oxyde d'étain, le chlorure stanneux ou l'association des deux, le stanoxyl disodique, le dibutylmalléate d'étain et le dibutylmalléate-dibutylétain. Ces produits ont été administrés par voie orale, sous-cutanée, intrapéritonéale ou intraveineuse chez des Rats et des Lapins normaux ou parasités pendant une période variant de quelques jours à six mois. Les médicaments stanniés

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étaient suivis dans l'organisme par leur localisation histochimique et leurs effets histopathologiques sur les tissus et organes. Les résultats des localisations histochimiques, ainsi que l'observation des coupes et des animaux au cours des traitements mixtes ont permis d'apprécier la fonction, l'effet thérapeutique possible de ces produits en rapport avec leur nature (insoluble, soluble, minérale, organique), leur degré d'ionisation (plus ou moins résistable) et leur mode d'administration.

L'examen des résultats au niveau des lieux d'introduction, des voies de transit, des lieux de fixations et des points d'élimination peut donner quelques renseignements utiles.

Nous avons surtout examiné les tissus et organes où les dérivés de l'étain ont joué leur rôle thérapeutique antiparasitaire intra- et extra-intestinal en particulier au niveau du tractus digestif, du foie, de la rate, des reins, du poumon et des vaisseaux, leur effet antipsoriasique au niveau de la peau et des phanères, et enfin nous avons donné leur effet toxique lointain au niveau du cerveau.

Dans le tractus digestif au niveau de l'estomac et de l'intestin nous avons examiné l'effet de l'oxyde d'étain et du chlorure d'étain en poudre, seul, ou associés, ajouté à l'alimentation. Administrés par voie orale les dérivés stannés passent assez rapidement dans ces organes : l'étain se retrouve au niveau de la muqueuse de l'estomac, dans les replis et les lumières des glandes et surtout au niveau de la muqueuse intestinale. Ces muqueuses sont fortement colorées par les réactifs montrant ainsi une localisation massive. Dans l'estomac le produit paraît passer sans être résorbé, dans l'intestin la résorption est plus grande, mais l'observation cytologique est très malaisée. On note que la muqueuse est fortement affectée par la caustricité de ces dérivés stannés. Dans les cas d'intoxication, l'estomac et l'intestin perdent leur tonus et leur élasticité, se dilatent facilement, montrent de nombreuses ulcérations. L'étain minéral et organique, lorsqu'il est légèrement ionisé, peut franchir la barrière intestinale et se retrouve dans les vaisseaux de l'intestin, dans le foie, dans la rate, les reins, les poumons, etc... Le degré d'absorption varie avec la nature du produit administré. L'étain métallique est peu actif, l'étain minéral insoluble (oxyde et chlorure), mais soluble, traverse la barrière intestinale. Il en est de même des dérivés solubles minéraux et organiques qui vont beaucoup plus loin puisqu'on les retrouve dans le système nerveux, dans la peau, les poils et dans les divers émonctoires. Administré par voie parentérale nous n'avons pas retrouvé l'étain dans les tissus et muqueuse de l'estomac et de l'intestin. L'activité antiparasitaire intra-intestinale est liée à la causticité des sels d'étain, à l'effet sur les protéines des parasites et de leurs enzymes. L'étain, pour avoir une action efficace sur les parasites intestinaux, doit être légèrement ionisé et peu soluble ; à dose égale les sels minéraux seraient les meilleurs avec une toxicité moindre. Le dibutylmalléate d'étain, organique et insoluble, est actif par la voie orale. Plus résorbable il présente des actions extra-intestinales.

Dans le foie du Rat et du Lapin, on retrouve l'étain en quantité notable et sa présence peut expliquer l'activité sur des parasites intra-hépatiques. Les dérivés minéraux peu solubles (oxyde anhydre ou hydraté) ou organiques (dibutylmalléate) à des doses convenables sont résorbés par la muqueuse et passent dans le foie. Ils peuvent, à faible dose et avec une durée convenable, réaliser une imprégnation hépatique supérieure à celle obtenue avec les dérivés solubles minéraux et organiques administrés par la voie parentérale. Administrés à forte dose ils ne peuvent pénétrer dans le foie que si le système de défense est détourné ; par contre à faible dose

et lentement ils peuvent pénétrer dans le parenchyme hépatique et avoir une certaine activité. Ces résultats des localisations hépatiques expliquent les succès observés dans le traitement des bilharzioses et distomatoses.

Dans les poumons de ces mêmes animaux, les dérivés minéraux, solubles et insolubles injectés peuvent pénétrer dans le parenchyme pulmonaire où ils sont fixés et éliminés par les cellules nucléées et les cellules fibres de la cavité pulmonaire. Les dérivés organiques comme le dibutylétheylétain, plus solubles et plus toxiques, par la même voie pénètrent moins ; on les retrouve cependant dans les vaisseaux plus ou moins congestionnés ; ainsi véhiculés ils traient plus loin et intéressent dans le système nerveux. Chez nos animaux les dérivés minéraux administrés *per os* ne sont retrouvés que plus faiblement dans les poumons et au cours d'un traitement plus prolongé. La présence de l'étain dans le parenchyme peut expliquer le pouvoir thérapeutique de quelques dérivés stannés dans le traitement de la grippe et des affections pulmonaires, en particulier de la tuberculose.

Dans la peau et les phanères les dérivés de l'étain par administration sous-cutanée ou orale sont retrouvés, on les observe dans le tissu sous-dermique, dans les muscles, dans les vaisseaux et surtout dans les follicules pileux. L'étain est éliminé par la peau et les poils, on le retrouve en effet dans le bulbe, dans la gaine épithéliale interne et dans les cellules de la moelle du poil selon la durée de l'administration. Cette présence dans la peau et surtout au niveau des follicules pileux expliquerait l'activité thérapeutique des sels d'étain dans le traitement de la teigneuse et des autres à staphylococques.

Dans le cerveau les dérivés minéraux peuvent se retrouver à l'état de traces dans les vaisseaux de la pie-mère : la voie parentérale donne une plus faible localisation que l'administration *per os*. L'étain organique, comme le dibutylétheylétain, en injection intraveineuse, peut être retrouvé dans les vaisseaux de la pie-mère, dans les vaisseaux intra-cérébraux, au niveau de la substance blanche ou grise. Avec des fortes doses dans la région moyenne de l'écorce grise, au voisinage des vaisseaux on peut trouver quelques cellules pyramidales plus ou moins altérées présentant dans leur protoplasme des traces diffuses d'étain. Bien que l'étain soit fortement combiné à des protéines, on note exceptionnellement des signes d'intoxication de type nerveux et l'altération des cellules nerveuses. On peut concevoir que le dibutylétheylétain et les sels organiques de ce genre soient toxiques et expliquer ainsi les accidents thérapeutiques observés. La solubilité de ces produits stannés organiques dans les lipides, comme nous l'avons vu avec Gatanis, dans les cellules du poumon, peut aussi contribuer pour une part à éclairer l'action neurotrope de ces substances sur le cerveau riche en lipides.

RÉSUMÉ

Après avoir rappelé les techniques de détection histochimique de l'étain sous ses différentes formes, minérale, organique, soluble ou insoluble, réaction au dithiol, réaction à l'hydrogène sulfuré ou solution métallique, technique des lignes tirées au pinceau au Laboratoire d'histologie de Montpellier, les auteurs ont étudié l'effet de la localisation de quelques médicaments stannés

comme l'oxyde d'étain, le chlorure stanneux ou l'association des deux, le stannoxyl dissous, le dibutyldilaurate d'étain et le dioctodiéthylétain chez des Rats et des Lapins normaux ou parasités et cela à divers niveaux : tractus digestif, foie, vaisseaux, rate, reins, poumons, peau et phanères, cerveau. Dans ce dernier organe l'étain se retrouve non seulement dans les vaisseaux pie-mériens et intra-rétiniens, mais encore combiné à des protéines et à des lipides à l'intérieur même des cellules pyramidales.

SUMMARY

After having recalled the histochemical detection techniques of tin in its various forms, mineral, organic, soluble or insoluble, reaction to dithiol, reaction to sulphuretted hydrogen in acetone solution, techniques of Inques perfected at the Montpellier histology Laboratory, the authors have studied the effect of localisation of a few stannated medicines like oxide of tin, stannous chloride or association of the two, dissolved stannoxyl, dibutyltinlaurate of tin and dioctodiethyl-tinnum on Rats and Rabbits, normal or parasitized at various levels, digestive tract, liver, vessels, spleen, kidneys, lungs, skin and annexes, brain. In the last organ tin is found not only in the pia-mater and intra-retinal vessels but also combined with proteins and with lipides in the interior itself of pyramidal cells.

RESUMEN

Después de recordar las diferentes técnicas de pruebas histoquímica del estaño bajo sus diferentes formas, mineral, orgánica, soluble o insoluble, reacción al ditiol, reacción al hidrógeno sulfurado en solución acetónica, técnicas de Inques perfeccionadas en el Laboratorio de Histología de Montpellier; los autores han estudiado el efecto de la localización de algunos medicamentos estánnicos, como el óxido de estaño, el cloruro estanoso o la asociación de ambos, el stannoxil disuelto, el dibutilfilaurato de estaño y el dioctodietilestanno, en ratas y en conejos normales o parasitados, a diversos niveles: tracto digestivo, hígado, vasos, bazo, riñones, pulmones, piel y faneras, cerebro. En este último, el estaño se encuentra no sólo en los vasos piamaterales e intra-retinales sino hasta combinado con proteínas y lípidos en el interior mismo de las células piramidales.

OUTBREAK OF FOODBORNE ILLNESS ATTRIBUTED TO TIN

Sherman Warburton, M.P.H., Wallace Giles, Raymond M. Ewert, M.P.H., and William S. Hayes, M.D., M.P.H.

AN OUTBREAK of gastrointestinal distress among a party of women who attended a bowling league banquet led to identification of a fruit punch as the probable cause. Investigators found a high concentration of tin in the punch which they concluded had resulted from improper storage of the relatively acid beverage in a 5-gallon milk can which had been recently refined. Although tests for copper, zinc, and cadmium were negative, the possibility that other toxic chemicals were present in the refined can was not eliminated.

On the evening of April 18, 1961, the Denver Department of Health and Hospitals referred to the Tri-County District Health Department in Aurora, Colo., a report from the local Air Force base infirmary of several cases of "food infection" or "food intoxication." Contact with the infirmary confirmed the report. The patients, who had attended a noon banquet that day at a local restaurant, had been treated for nausea, vomiting, and diarrhea.

The Investigation

A sanitarian from the health department was immediately dispatched to the restaurant to make a preliminary investigation. He ascertained the complete menu served at the banquet and obtained samples of all leftover foods. He also determined the type of food service, the number of people served, names and addresses of persons attending the banquet, sources of food and food products served, and names and addresses of all food-handlers and other employees involved.

The investigation revealed that 85 persons and guests of a nearby Air Force officers' Wives Bowling League had attended the banquet. The food was served buffet style. In most instances, the food was satisfactorily protected from contamination, with adequate facilities for icing the cold foods and a steamtable to keep the hot foods hot.

The following morning, members of the bowling league were interviewed, and a complete list of all persons attending the banquet was obtained. A questionnaire, drafted to facilitate tabulating the statistical data needed, was distributed to every person attending the banquet.

Thirty-eight women attended the banquet, and 37 filled out the questionnaire. Thirty-one of the 37 (83.8 percent) reported illness, and 6 (16.2 percent) reported that they were not ill. One questionnaire was not tabulated because of unusual circumstances.

Onset of illness was described as rather abrupt. Typically, the first symptom was a burning feeling followed by an almost overwhelming nausea, stomach cramps, and vomiting. In the evening, approximately one-third of the patients had a mild diarrhea. The high percentage of patients with nausea, abdominal cramps, and vomiting (table 1), along with a short incubation period, strongly indicated that the illness was caused by a chemical or bacterial poison, rather than a bacterial infection or intoxication.

Figuring the incubation period from the time the patient reported eating until she first noticed symptoms, the shortest period was "less than 1 hour" and the longest was 2 hours. Twenty women reported that they first noticed symptoms during eating or immediately after eating, and four said it might have been 2 hours from the time they started eating until they noticed symptoms. A few indicated slight distress over

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Table 1. Frequency of symptoms among 30 persons who reported illness following the banquet

Symptoms	Number with symptoms	Percent with symptoms
Nausea	29	96.7
Abdominal cramps	26	86.7
Vomiting	21	70.0
Diarrhea	17	56.7
Chills	14	46.7
Diaphoresis	10	33.3
Blurred vision	5	16.7
Fever	4	13.3
Tremor	3	10.0
Sore throat	3	10.0
Pain	2	6.7
Rash	1	3.3

before eating, and after eating a small amount, they had to be excused from the table because of extreme nausea. Because of this unusual condition, it was impossible to figure the mean interval between ingestion and distress.

The illness lasted an average of 15.7 hours. The shortest period of illness was 2 hours; the longest, 48 hours. Seventeen of the 30 women reported that they had recovered within 12 hours, but the remainder reported ill effects for a longer time. Six of the women reported seeing a physician.

Attack rates for the foods served at the banquet indicated that the vodka punch, served before the dinner, and the potato salad were the items most likely to be responsible for the illness (table 2). One woman who did not drink punch reported illness. She was interviewed carefully, and it was learned that she was the only one who developed a rash, and that she was one of three women who reported sore throat and fainting or dizziness. As these symptoms were not characteristic of this outbreak, it is highly probable that her illness was not associated with the banquet food.

Laboratory Findings

Samples of beef, chicken, scallops, gravy, tartar sauce, potato salad, macaroni salad, kidney bean salad, vodka, and fruit punch were submitted to the Colorado State Department of Public Health for laboratory analysis. Attack rates on specific food items and other statistical information which suggested that the outbreak might be due to a chemical poisoning or irritant were reported to the laboratory.

Laboratory analyses of the foods failed to establish evidence of any bacterial contamination which would be pathogenic. Chemical analysis, however, revealed that the fruit punch used as a base for the vodka punch was contaminated with 2,000 mg. of tin per liter of punch. The

Table 2. Attack rates for specific foods served at the banquet

Food	Did eat			Did not eat			Difference
	Total number	Number ill	Attack rate	Total number	Number ill	Attack rate	
Beef and wine	19	15	78.9	17	15	88.2	9.3
Pot roast	11	9	81.8	25	21	84.0	2.2
Baked chicken	26	23	88.5	10	7	70.0	18.5
Scallops	28	25	89.3	8	5	62.5	26.8
Gravy	19	15	78.9	17	15	88.2	9.3
Tartar sauce	10	9	90.0	26	21	80.8	9.2
Baked beans	8	8	100.0	28	22	78.6	21.4
Boiled cabbage	13	10	76.9	23	20	87.0	10.1
Potato salad	32	28	87.5	4	2	50.0	37.5
Macaroni salad	22	20	90.9	14	10	71.4	29.5
Kidney beans salad	16	15	93.8	20	15	75.0	18.8
Lettuce salad	25	22	88.0	11	8	72.7	15.3
Cabbage slaw	25	22	88.0	11	8	72.7	15.3
Orange cheese	11	8	72.7	25	22	88.0	15.3
Fruit jello	12	10	83.3	24	20	83.3	0
Coffee	31	25	80.6	5	5	100.0	19.4
Tea	1	1	100.0	35	29	82.9	17.1
Milk	1	1	100.0	35	29	82.9	17.1
Vodka punch	32	29	90.6	4	1	25.0	65.6

laboratory found no evidence of copper, zinc, or cadmium.

No stool or vomitus samples were obtained because at the time of the investigation all vomiting and diarrhea had subsided. The laboratory staff reported that it would have been extremely difficult to isolate any toxins from the stool or vomitus. They suggested, however, that if the outbreak was caused by a chemical, the onset of illness was so violent that it was almost certain that the toxin was expelled.

Tinsdale (27) tentatively established minimum doses of soluble tin salts as the causative agent. Although such intoxication is exceedingly rare, ingested tin has occasionally been responsible for gastrointestinal irritation causing extreme nausea, vomiting, cramping, and diarrhea (27). The concentration of tin which is toxic has not been determined. Dack (28) cites an experiment in which four subjects ate canned pumpkin containing 383–476 ppm of tin and canned asparagus containing 301 ppm of tin for 6 days with no illness. There is no hazard in the commercial packing of food in tin-lined containers under prevailing practices.

Vehicle of Transmission

The fruit punch implicated as the vehicle of transmission was purchased by the restaurant from a local dairy. Although there was no obvious fermentation, the punch, made from reconstituted pineapple-grapefruit juice, had a pH of approximately 3, indicating a relatively high acid. It was delivered to the restaurant in a returned 5-gallon milk can at approximately 10 a.m. the day of the banquet. On delivery, the can was placed in a walk-in refrigerator and stored until the next noon. The punch was then transferred to a punchbowl and prepared to serve at approximately 12 o'clock noon.

The health department was unable to obtain information on when the punch was processed or how long or in what kind of container it was stored before delivery. If it had been permitted to stand in an open tin-lined container, oxygen would have contributed to reaction of the acid with the tin lining.

The milk can in which the punch was delivered showed obvious evidence of corrosion. A distinct line of demarcation was evident at the liquid level. Scrapings taken from the bottom and sides of the can with a sharp instrument indicated that the lining contained large amounts of tin, as determined by hydrogen sulfide precipitation and tetraphenylarsonium confirmation.

Summary and Conclusions

On April 18, 1961, the Tri County Health Department, Aurora, Colorado, was notified of a possible food infection or food intoxication originating at a suburban restaurant. An investigation revealed that 38 members and guests of a bowling league had attended a banquet at the restaurant and that during dinner and immediately after the meal 31 of the persons attending were stricken rather suddenly with a severe gastrointestinal irritation.

Clinical symptoms, manner of onset, attack, recovery, duration of illness, and attack rate indicated that the causative agent might have been a chemical toxin or irritant rather than a bacterial infection or intoxication. Attack rates for the food items served implicated the fruit punch, and laboratory analysis verified the epidemiological inference. The punch was found to be contaminated with 2,000 mg. of tin per liter of punch. Details concerning handling of the punch were not available; however, it was established that it was delivered to the restaurant in a returned milk container.

On the basis of these findings, it appeared that the illness was caused by tin poisoning, although other toxins may have been present in the lining material of the milk can. It was assumed that the contamination of the punch with tin was the result of improper handling, particularly storage and shipment in an improper container. It seems probable that the unusually high acidity of the punch (pH 3) caused reaction with the lining of the container.

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