THE TOXICOLOGY OF TIN COMPOUNDS

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Tin and its alloys played an important part in man's emergence from the Stone Age and have since made valuable contributions to the economy in peace and war. However, from a biological point of view the salts and compounds of tin have excited little interest when compared, for example, with those of mercury, lead or iron. The human body contains very little tin (27, 67) and no one has yet demonstrated that it is an essential element in any biological system. No critical review of the biological activities of tin compounds could be found, but the recent commercial exploitation of several alkyl tin derivatives coupled with an appalling disaster in France caused by the inclusion of some of these compounds in a remedy for skin sepsis provided a stimulus to review the existing information.

However, for the reasons mentioned above, there is comparatively little material to review and much of it is so sketchy in quality and range as to make the drawing of conclusions hazardous and possibly misleading. After noting briefly the uses of tin compounds in the biological field, the properties of the inorganic and organic compounds are reviewed. In recent years particular attention has been paid to the trialkyl tin compounds and a large part of this review describes some of the toxic properties of this interesting group of compounds.

USES

Bactericidal action. Even in these days of powerful antibiotics the belief that tin and its compounds have some value in the control of cutaneous sepsis persists. Prompted by the traditional belief that the tin workers of Beauve never suffered from boils and that tin powder was a popular remedy for such a condition, Frouin and Grégoire published in 1917 their experimental and clinical evidence that tin compounds modified bacterial infections (34, 35, 40). This evidence, based as it was on a study of 7 infected rabbits with 2 control animals, claimed to show that tin, tin oxide, stannous chloride and sodium stannate modified the virulence of staphylococci but only delayed death in infected rabbits. How it ever came to be given serious consideration must remain a mystery of medical mythology. The belief has remained despite later evidence that neither the soluble nor insoluble compounds of tin have any effect against staphylococci either *in vitro* or *in vivo* (57, 79). There is no trace of any critical clinical trial of the value of tin in furunculosis. A claim that tin and stannous oxide might benefit cases of typhoid fever excited no more attention than the evidence deserved (78).

The faith in the virtues of the insoluble tin compounds such as stannous oxide at least did the patient no harm, but the idea that the soluble organic tin compounds might prove to be more active led to the French tragedy.

"Stalinon". "Stalinon" was a proprietary preparation sold in capsules throughout France for the treatment of furuncles and other staphylococcal skin infec-

tions, osteomyelitis, anthrax and acne. It was said to contain diethyltin di-iodide (15 mg per capsule) and "Vitamin F" (linoleic acid) (100 mg per capsule). The recommended dose was 6 capsules a day for 8 days for the adult and half that amount for children. Two hundred and seventeen people are known to have been poisoned by this preparation and one hundred of these people died. The total number of people who took these capsules is not known but is believed to be about one thousand. A full report of the legal proceedings which followed this episode has been published (77) and clinical and pathological information on the poisoned cases is given in the following papers (1, 20, 31, 32, 33, 37, 41, 42, 58, 76, 81, 82, 83).

During the period when the cases were occurring the composition of the "Stalinon" capsules varied. The main impurities were monoethyltin and triethyltin. The latter which is 10 times as toxic as diethyltin to rats by the oral route (8) contributed up to 10% of the theoretical amount of diethyltin. An estimate of the toxic dose for an adult would be about 70 mg triethyltin spread over 8 days (Dr. R. LeBreton, personal communication).

Alajouanine, Derobert and Thieffry (1) have surveyed the clinical data in 201 of these cases. Ninety-eight patients in this group died. Symptoms of poisoning appeared after a latent period of about 4 days. The most constant symptom was severe, persistent headache. Other common symptoms were vomiting, retention of urine, vertigo, abdominal pain and visual disturbances, especially photophobia. There was a rapid loss of weight. Psychic disturbances were common and the presence of these symptoms was one of the best indications of the severity of the case. Pyrexia was usually absent and several cases showed hypothermia (34°C). Even in fatal cases physical signs were often absent and when present they comprised signs of meningism, transient pareses and sometimes permanent paralysis. Papilloedema was sometimes seen. The cerebrospinal fluid was usually normal in composition but its pressure was raised (Dr. S. Thieffry, personal communication). The electroencephalogram was altered but not in any regular way and the changes did not suggest any localized lesion. Death occurred either in coma or from respiratory or cardiac failure. In a few cases it occurred during the convulsions which were seen in some of the more severely affected patients. Of the 103 patients in this group who survived only 10 recovered completely and convalescence was very slow (Dr. S. Thieffry, personal communication). In the remainder symptoms such as asthenia and attacks of headache have persisted after 4 years and in some cases partial paresis, areas of anaesthesia and diminished visual acuity have remained. In 4 cases where there was severe flaccid paraplegia, incontinence and loss of sensation, these symptoms have persisted unchanged. The only treatment which seemed of any benefit in the acute stage was surgical decompression and in some cases this appeared to be a life-saving measure. The autopsy reports from these cases are rather scanty but in the 4 cases examined histologically (42) there was a striking interstitial oedema of the white matter which appeared quite unlike the cerebral oedema produced by other agents. There was no Wallerian degeneration and the only other lesion of possible significance was some endothelial proliferation in the smaller veins accompanied in

some cases by thrombosis and small perivenous haemorrhages. The cerebral oedema was also seen at operation in those cases subjected to decompression (26, 83).

Anthelmintic action. By tradition tin and its oxide have had a reputation as anthelmintics (71). Claims (36, 39, 41a, 45, 103) for the value of tin, tin oxide and stannous chloride in treating cestode infection in man appear to have the support of authority (11). The greatest success of stannous oxide appears to have been achieved by combining it with an antimalarial "amodiaquine." This kills or weakens the taeniae while the mild purgative action of 25 tablets of 100 mg of stannous oxide hastens their physical removal from the gut (41a). Clinical evidence has received some support from experimental observations on rats. While tin and its inorganic compounds were ineffective against *Rhabditis macrocera in vitro*, they were active against cestodes though not against nematodes in rats and mice. The organic compounds—particularly triethyltin—were, on the contrary, very active *in vitro* but ineffective *in vivo* and killed the host preferentially to the parasite (12, 39). How the insoluble tin compounds can dislodge cestodes from the alimentary tract is not known. Existing information on the toxicology, biochemistry or pharmacology of these inert materials offers no basis for a working hypothesis.

Although the organo-tin compounds proved too toxic for the cestode-infected rat, the dibutyltin compounds have been shown to have a very effective action on the hen where the margin of safety (therapeutic index) is quite adequate (53). Administered either as a single dose (100 mg/kg) or incorporated into feed (300 ppm [parts per million]) dibutyltin salts were effective against *Raillietina cesticillus* (54, 55). Although many salts of dibutyltin were equally effective, neither the other dialkyl homologues nor the tri- or tetraalkyl tins had any effective therapeutic action in the hen (55). Dibutyltin dichloride was also effective against *Hymenolepis fraterna* in the rat (12).

No explanation for the relative insusceptibility of the bird to the toxic action of dibutyltin salts can be offered. However, the biochemical activity of these compounds as well as their toxicity to other species is well established (see below). There is every reason to discourage their clinical trial as anthelmintics despite a suggestion to the contrary (11).

Fungicides. Though reported to be an excellent preservative for anatomical specimens (99) the salts of tin, unlike those of copper or mercury, for example, have no practical value as fungicides. A systematic study of the alkyl tin compounds, however, indicated that trialkyl tin salts were extremely effective against several fungi when tested *in vitro* (50). The mono- and tetraalkyl tins were ineffective and the diethyltin very much less active. The nature of the salt made little difference to the activity of triethyltin as a fungicide but the tributyl and tri-*iso*-propyl tins were slightly more effective while the trimethyl and triphenyl were slightly less so. The trioctyl salts were very much less active. It was the total number of carbon atoms attached to the tin rather than the size of any individual substituent that determined the activity of the compound as a fungicide. The optimal number was 9 to 12 carbon atoms (51).

The lower volatility and slightly greater effectiveness of tributyltin salts may make them more popular than those of triethyltin as fungicides for many commercial purposes, such as timber preservatives, and as additions to wood pulp baths and paints (95). Though less toxic to rats than the triethyltin salts either as a single dose or by feeding (8) there is not yet enough information to enable it to be said that the organotin fungicides are significantly less hazardous in use than the other main group of effective eradicant fungicides—the organomercury group. Although the animal experiments suggest that the action of the trialkyltins is reversible while that of the alkyl mercurials is not (47), the clinical observations on the "Stalinon" victims suggest that permanent injury may sometimes be suffered by man (1).

The first organotin compound to be introduced as a fungicide in agriculture was a preparation of triphenyltin (43). This has proved effective on certain crops, but there is little information available on its toxicity to mammals; the LD50 for rats is stated to be 125 mg/kg (43).

Insecticidal action. Although patents for organotin compounds as moth-proofing agents have existed for some years (50) none has been commercially developed. Triethyltin compounds have been shown to be very toxic to flies (9) but it seems improbable that they could be exploited for large-scale or domestic use for this purpose.

Antitumour action. Several organotin compounds tested against tumours in mice were found to be ineffective (19).

Industrial uses. It is as stabilisers incorporated into certain plastics (52) used to make tubing or film for wrapping food, that the dialkyl tin compounds might give rise to a potential health hazard to consumers. Information on the amounts, if any, of these stabilisers that can leak out under conditions of use has not been published.

BIOLOGICAL PROPERTIES

Metallic tin and its salts. The relative resistance of tin to the action of dilute acids, particularly under anaerobic conditions, makes it a suitable lining for the cans used in food preservation. The knowledge that such food might be contaminated by tin stimulated some earlier investigations into its toxicity (28, 59, 87, 101, 104). The degree of contamination depends on the contents, type of can and conditions of storage (6). The unofficial maximum permitted level in the United Kingdom is 250 ppm at which stage, in most cases, the food would be unpalatable because of the amount of iron also dissolved from the can. Studies of the contents of cans stored for long periods showed that very high levels of tin could be reached; 2,440 ppm in a can of carrots and gravy after 114 years were reported (46). Up to 2,000 ppm have also been found in cheese (28). However, it seems more probable that bacteria rather than an excess of the metal were responsible for some of the episodes of "acute food poisoning" described 50 years ago and attributed to tin.

Experiments attempting to demonstrate the toxicity of the simple tin salts are difficult to interpret because of the acidity and irritating properties of their

solutions (69, 71, 101, 104). Nevertheless, on the rather flimsy evidence available it seems to be accepted that the salts of tin are poisonous and that they produce paralysis and other neurological damage (30).

One of the most comprehensive studies of the toxicity of tin salts (101) was carried out with sodium stannous tartrate. Not only are solutions of this salt less acid than the simpler salts, but it was thought a tartrate might be a product of any reaction taking place between the fruit and its tin container. Nevertheless in these experiments the solutions were given by subcutaneous injection and not by mouth. Though frogs, cats, dogs and rabbits were used, the greater part of the work was done on rabbits which received daily subcutaneous injections with the following results:

Daily dose (mg/kg)	12.5	5.5	3.8	3.0	2.4	1.6	1.0	0.5
Days before death	6	18	48	42	124	255	—	—

Diarrhoea often occurred in the early stages but death was always preceded by paralysis accompanied by twitching of the limbs though convulsions were not observed. Dogs treated in the same way also developed paralysis with no evidence of any sensory involvement. Cats were more sensitive than dogs or rabbits and developed paralysis with anaesthesia of the limbs. Cats and dogs vomited after the larger doses (86, 101). No suggestions were made as to the site of action of the sodium stannous tartrate within the central nervous system and no pathological lesions were described. These findings confirmed earlier work (104) and more recent observations on rabbits have further substantiated the main finding (86, 97).

It has been alleged that stannous tartrate when injected intravenously in rabbits will produce albuminuria (85) but other workers have claimed that the toxicity of stannous tartrate or stannous citrate when injected into rabbits in single doses was no different from that produced by the equivalent sodium salts (70, 93).

When these compounds are injected parenterally the tin is widely distributed in the body at first and then the tissue concentrations gradually fall except in the liver and spleen where the level increases before it decreases (39, 93, 97). It would seem that when these compounds are injected they are quickly converted to a particulate form which is removed by the cells of the reticulo-endothelial system.

Pathological changes are seen especially in the spleen and in the liver. The spleen has a bluish-grey appearance due to the deposition of the injected tin in the reticulo-endothelial cells (59, 97). Ultimately the tin is excreted through the kidneys with the bile as a minor pathway (49, 87). The belief that the saliva was an excretory pathway (100) is in conflict with older evidence based on chemical analysis (87). Although the brain contained more tin than the liver or kidney after intravenous injections (104) there was less in the brain after stannous tartrate was given by mouth even when the animals were paralysed (28).

When an attempt was made to reproduce the nervous condition by giving tin by mouth, a single dog given stannous chloride in milk did eventually develop

paralysis after 14 months when the daily dose was 500 mg/kg (101). However, the evidence that sodium stannous tartrate is toxic by mouth is more equivocal (28, 84, 101) except perhaps when very large doses are given (101). Absorption of inorganic tin from the alimentary tract in man (39, 87), the dog (87), the cat (59), the rat (39) and the rabbit (28) was certainly poor, although small amounts of metallic tin, its inorganic salts and such compounds as sodium tin tartrate may pass into the tissues of these species (39, 87). Even in the case of the soluble sodium tin tartrate more than 90% of the dose was recovered from the faeces (87). Tin was adsorbed by protein thus inhibiting its digestion by proteolytic enzymes (38). This may be a factor in the poor absorption of tin. The small amounts which are absorbed are found mainly in the liver and kidneys with traces in other tissues (28, 39, 56).

Although rats fed on a stock diet cooked in Indian vessels (turned brass) did not grow as well as those getting a diet prepared in glass vessels (25), the data provided is not adequate to assess the significance, if any, of this observation. Three cats survived in normal health for 390 to 612 days on a normal diet to which was added daily up to 40 mg/kg tin as a simple organic salt (59) and guinea pigs thrived for 4 months on a diet containing 770 ppm tin salts (91). In neither the cats nor the guinea pigs was tin found in the organs. Powdered tin given to rats and pellets of tin given to hens proved completely harmless in contrast to metals such as lead, bismuth, cadmium or nickel given in the same way (44, 102).

When tin is taken into the lungs either as dust or fumes it leads to a benign pneumoconiosis. This condition is symptomless and does not interfere with function. More than 150 cases of this condition have been described in the literature (24, 27, 72, 75, 80, 89); these were discovered largely as a result of X-raying workers in tin foundries. The gross X-ray appearance is due to the high atomic weight of the tin giving a very radio-opaque shadow. In a case (27) which died of intercurrent disease the lungs contained 110 mg tin per 100 g wet wt. and X-ray diffraction patterns showed that it was in the form of SnO_2. There was no tissue reaction to the dust in the lungs and this was also found when it was injected intraperitoneally in guinea pigs (72). The dust was deposited as nodules, the particles being mostly extra-cellular with some in macrophages. There was no necrosis, foreign body giant cell reaction or collagen formation.

To mice and guinea pigs tin hydride (SnH_4) was more toxic than arsine but its effects appeared to be mainly on the central nervous system and no haemolysis was produced (73). The reaction of the spinal cord of frogs to tin salts (68) and the absence of any effect of these salts on tadpoles (90) appear to represent the sum total of classical pharmacological observations on these inorganic compounds.

Organic tin compounds. Consideration will be limited to compounds of the following general categories: R Sn X_3; R_2Sn X_2; R_3 Sn X; R_4Sn.

R is a simple aryl or alkyl group linked direct to the tin and X a simple or complex anion. The preparation and general chemistry of these compounds have been described (52, 61). The biological properties of each group are distinct and, within

any group, vary with the nature of R and are almost independent of the nature of X.

It is just over 100 years ago that Buckton (10) described the preparation of triethyltin chloride and noted its nasal irritant properties and ability to produce headaches. This property led to organotin compounds being screened as possible sternutators (63). Buckton's observations were confirmed 11 years later by Jolyet and Cahours (48) who distinguished the diethyltin dichloride with its general irritant and emetic action on the gut from the more toxic triethyltin which in the frog and dog led to progressive weakness and paralysis. However, they noted that complete recovery could follow even after a dog had had convulsions. They also found that tetraethyltin produced in the frog and dog the same effects as the triethyltin chloride. Work since then has only added to or elaborated upon these fundamental observations on the properties of this group of compounds.

Monoalkyl tins. Little is known about these compounds. A few observations with monoethyltin trichloride suggested that its toxicity was low (97).

Dialkyl tins. After the early reference to the irritative action of diethyltin (48) little has appeared on the toxicity of this group of compounds. Diethyltin dichloride has toxic effects on rats that are quite distinct from those of the triethyltin though the picture of poisoning lacks any prominent features. 2,3-Dimercaptopropanol (BAL) is an effective antidote (97). All the dialkyltins from methyl to hexyl were toxic to rats and the gradation was less sharp than in the case of the trialkyl analogues. Dioctyltin was not found to be toxic to rats, mice or guinea pigs in oral doses up to 400 mg/kg given for 3 to 4 successive days nor were any ill effects found when it was added to the diet of rats for 4 months at 200 ppm (8). Toxic effects with this compound have been reported after single oral doses of more than 690 mg/kg in the mouse and 920 mg/kg in the rat; the intraperitoneal toxicity was about 10 times the oral toxicity so that death of mice and rats could always be produced by doses of 20 mg/kg dioctyltin diacetate repeated every other day for 30 days (66). The dibutyltin salts, and to a lesser extent the other analogues (diethyl to di-*n*-hexyl), produced in rats and mice but not in rabbits or guinea pigs a lesion of the bile duct which may cause death of these animals from liver failure or peritonitis (7). Dibutyltin salts did not produce bile duct lesions either in the hen (55) or in the cat (Barnes, unpublished observations). After the injection of diethyltin and dibutyltin the highest concentration of tin was found in the liver with a smaller amount in the kidneys (7, 39). For dibutyltin the bile was one route of excretion through which it passed unchanged (7).

In the rat pulmonary congestion and oedema were observed after the intravenous injection of solutions in 0.05 ml polyoxyethylene sorbitan mono-oleate (Tween 80) of the diethyl, di-*n*-propyl, di-*iso*-propyl and di-*n*-pentyl tin compounds and adrenal haemorrhages were sometimes found after the injection of the higher members of the series (8). The most characteristic pathological findings were in the biliary tract; these have been described in detail (7).

In the rat shortly after the administration of dibutyltin an inflammatory

lesion appeared in the wall of the bile duct at a point just before it enters the duodenum. This led to sloughing of the lining epithelium and often perforation of the duct. There was a pancreatitis confined to the part around the duct. The duodenum was unaffected. The lesion was reversible but after repeated doses the wall of the lower part of the bile duct became permanently thickened with extreme dilation above and fibrosis of the surrounding pancreas. The inflammatory process extended upwards along the portal tracts into the liver giving hepatic necrosis and at times the formation of wedge-shaped infarcts.

Similar changes occurred in mice but dibutyltin had no effect on the bile ducts, liver or pancreas of rabbits or guinea pigs or cats. Such lesions have not been reported after diethyltin di-iodide in man. These species differences may be related to differences in the anatomy of the bile and pancreatic ducts. No renal lesions were seen in any of these species. Although BAL protected rats against the general toxic effects of the dialkyltins it had no influence on the severity of the biliary lesions. The mechanism of the lesion is not known but two facts may provide a clue. Dibutyltin is excreted in the bile and the lesion is seen only in those species where the bile and pancreatic ducts have a common course.

Long-term administration of dibutyltin to rats produced no lesions in addition to those seen after single doses (8). The lower homologues in the series of dialkyltin dichlorides caused severe lesions when applied to the skin of rats and guinea pigs (8) but the dipropyl and higher homologues caused relatively little damage. The dibutyltin dichloride but not its other salts caused mild skin lesions in man (62).

Biochemical effects of dialkyltin. There have been relatively few studies of the biochemical effects of the dialkyltin compounds. It does not cause cerebral oedema. In acute experiments on the rat (64) diethyltin had no effect on the sodium and potassium contents of the brain and spinal cord and caused a small decrease in their water content.

Studies with rat brain brei and liver mitochondria (3, 4) have shown that there was a clear distinction between the biochemical actions of the di- and trialkyltin compounds. The main action of dialkyltin was an inhibition of α-keto acid oxidases and in this it resembled phenylarsenious acid. When added to metabolising brain brei both inhibited O_2 uptake and led to the accumulation of pyruvate. When added to metabolising mitochondria both depressed O_2 consumption without lowering the P/O ratio. These effects were seen with the whole series of dialkyltin compounds up to and including di-*n*-hexyltin.

The similarity in behaviour to phenylarsenious acid is very close. The latter compound and others like it produce lesions of the biliary tract in some species. The biochemical effects of the dialkyltins and of these compounds can be prevented by BAL but not by glutathione. The great affinity of the dialkyltins for BAL can be demonstrated *in vitro* but there is only a slight reaction with glutathione. However, recent work has shown that although BAL completely prevented the accumulation of α-keto acids produced by the dialkyltins, these compounds appeared to differ from phenylarsenious acid in possessing another, as

yet unidentified, action in addition to their inhibition of α-keto acid oxidase *in vitro*. It remains to be seen whether this finding has any relevance to activity *in vivo*. (Dr. W. N. Aldridge, personal communication.)

Tetraalkyltins. The original observation (48) that tetraethyltin produced the same effect in animals as triethyltin has been confirmed (58, 97). Observations (105) on a few laboratory workers who handled tetramethyl and tetraethyltin showed them to have suffered from the same symptoms as the victims of "Stalinon" poisoning—headache and vomiting.

The actions of tetramethyltin have been briefly described (92); a whole series of these compounds has been investigated by Caujolle and his colleagues in mice and dogs (13–17, 60, 65). It was found that tetramethyltin was slightly less toxic than tetraethyltin which was the most active compound. For the higher members of the series the toxicity decreased with rising molecular weight. The tetra-*iso*-alkyl compounds were more toxic than the corresponding tetra-*n*-alkyl compounds. The signs of intoxication by tetramethyltin were different from those of the other members of the series: the picture was dominated by tremors and hyperexcitability, except after very large doses. The main effect of the other members was muscular weakness and paralysis leading to respiratory failure. With the tetra-*iso*-alkyl compounds this was often preceded by convulsive movements; closure of the eyelids and photophobia were described as constant signs. The authors emphasised the slow development of the signs of poisoning after the tetraalkyltin compounds, and drew particular attention to the number of delayed deaths. From the data they provide it can be seen that exceptionally a death occurred from 20 to 40 days after a single dose while the majority of deaths occurred during the first week. The similarity of these effects to those of the trialkyltin compounds is explained by the discovery (22) that the tetraalkyltins are biochemically inert until after their degradation to the trialkyltins, a reaction for which there is an active enzyme system in the liver (see next section).

Some of the pharmacological effects of these compounds in the dog have been described. When given intravenously in doses causing death in a few hours there was a progressive, although often biphasic, fall in blood pressure. Immediately after the injection there was often a period of apnoea followed by tachypnoea. Later, respiration became irregular and respiratory failure, which preceded cardiac failure, was the cause of death. The body temperature always fell and there was a rise in the blood sugar level (65).

It is difficult to decide to what extent these cardiovascular and respiratory effects should be attributed to pulmonary embolism. The compounds used (tetraethyl to tetraamyltin) are liquids insoluble in water and the precise nature of the injected material is not stated. The finding of pathological changes such as haemorrhagic oedema in the lungs of these animals suggests that the effects were in part due to pulmonary embolism.

Histological reports on the tissues of mice poisoned by tetraalkyltin compounds refer to a decrease in the cytoplasmic basophilia of the liver and, after tetramethyltin, tigrolysis of the Purkinje cells of the cerebellum (65). Oedema of

the white matter was not reported but an increase in the water content of the brain and spinal cord after tetraethyltin has been described (22) completing the analogy with the trialkyltin compounds.

As the tetraalkyltin is converted to the trialkyltin it is soon distributed in the same way as those compounds. In the rat shortly after the intravenous injection of tetraethyltin, triethyltin was found in the blood, liver, kidney and brain, in descending order of concentration. In the rabbit, however, the order was liver, kidney, brain and blood (22). The distribution of Sn^{113} labelled tetraethyltin and tetraamyltin has been studied in dogs and mice (16, 17, 65). Acute experiments on the dog showed that after the intravenous administration of tetraethyltin the radioactive tin was widely distributed in the body, being mainly concentrated in the lungs, liver and suprarenals. It was eliminated in faeces, bile and bronchial mucus and also to a variable extent through the kidneys depending on the degree of preservation of renal function. Similar findings were made with tetraamyltin and the localisation of the radioactive tin in the lungs was even more striking. A similar distribution was seen in mice. As the compounds were dissolved in Tween 80 and injected as an emulsion in physiological saline it is difficult to know if the large amount of Sn^{113} in the lungs after intravenous injection reflected the distribution of the Tween 80.

Metabolism of alkyltin compounds. Tetraethyltin was inactive when added to brain slices *in vitro*; however, when injected into the rat it produced, after a latent period, the same picture as triethyltin. Brain slices prepared from rats treated with tetraethyltin behaved in the same way as those from rats given triethyltin (22). Triethyltin soon appeared in the tissues of rabbits and rats given tetraethyltin and in amounts sufficient to account for the signs of poisoning. It would seem that tetraethyltin itself is an inactive compound effective only after its conversion to triethyltin. *In vitro* studies have shown that the most important site for this conversion was the liver where it was carried out by the microsome fraction plus the soluble fraction of the liver cell. A small amount of conversion could be performed by the kidney cells but other tissues such as the brain were completely inactive. The *in vitro* requirements for conversion were O_2, nicotinamide, Mg^{++} and triphosphopyridine nucleotide. The reaction was inhibited by 2-diethylaminoethyldiphenylpropyl acetate HCl (SKF 525-A). Female rats performed this conversion more slowly than male rats. The conversion of a tetraalkyl metal to a trialkyl metal has also been shown for tetraethyllead (23) and may be a general phenomenon.

Once tetraethyltin had been converted to triethyltin it appeared to persist in the body in that form. There was no evidence for the degradation of triethyltin or diethyltin after their injection into mammals.

Trialkyltins. The first extensive study of triethyltin acetate was carried out by White (104) as part of a study of the toxicity of tin. He used this compound to avoid the irritating effects of the simple tin salts. He stated that the toxic effects of triethyltin were not distinguishable from those of inorganic tin salts; this was in contrast to triethyllead which had an action quite distinct from that

of inorganic lead salts. (This point about the toxicity of the triethyltin as a radical is discussed below.) He gave a detailed account of the action of triethyltin acetate on the dog and rabbit and his observations have been summarized (88). Soon after injection the animals were sleepy for about 30 minutes—an effect attributed to the action of the organic radical itself. The animals recovered and 2 to 4 days later the signs of what was concluded to be tin poisoning appeared, with impaired movements of the hind limbs, general ataxia and unsteadiness finally leading to a complete paralysis.

White's conclusion is almost certainly incorrect. The trialkyltin ion is a stable entity which is toxic *per se* and persists for some time in the tissues (21).

The site of action of the triethyltin was not clear, but the signs of poisoning suggested brain damage. Full recovery could occur and no histological lesions were demonstrated. More recent work on rats and rabbits (97) has confirmed these earlier findings in animals poisoned by triethyltin. Progressive weakness and paralysis were seen in rats but rabbits frequently developed severe convulsions before death. The fowl, on the other hand, either showed no reaction or collapsed immediately after an intravenous dose. It might die or remained unconscious for an hour or so and then recovered completely. Late paralysis was not seen in this species. Whereas in the rat triethyltin was equally toxic by mouth or by injection, in the hen it is not toxic by mouth (97).

Given to rats in their diet triethyltin at 10 to 20 ppm produced a slow progressive weakness and loss of weight mainly accounted for by a failure to eat. Complete recovery could usually take place even from the most severe degree of poisoning but in a few animals chronically poisoned in this way tremors developed at a late stage and recovery from these was not observed (64). The similarity of the response to triethyltin in the different mammalian species tested experimentally has also been shown to extend to man in the cases of "Stalinon" poisoning (1).

Trimethyltin salts had the same order of toxicity as the triethyl ones and in rats death occurred up to 8 days after an average lethal dose. This was the longest interval between injection and death which was found (8). The delayed deaths reported after the tetraalkyltin compounds (13, 14, 15, 65) were not seen after the trialkyl derivatives (8). However, before the weakness and paralysis set in the animals went through an excitable phase sparring with one another and then developed severe persistent tremors (97). The same picture was seen with tri- and tetraethyl lead (23, 105). As the series ascended—*n*-propyl, *iso*-propyl, *n*-butyl and *n*-hexyl—the trialkyltins became rapidly less toxic to rats when given by mouth (8); although there is some disagreement on the relative toxicities of the tri-*n*-propyl and tri-*iso*-propyl compounds (18). Characteristic signs of poisoning were not seen though brain oedema had been observed with the tripropyl and tributyl tin salts. Trioctyltin was not toxic by mouth (8). The insolubility of the higher homologues makes comparison by intravenous injection difficult. Deaths either occur rapidly or not at all after injection by this route (8). Although tributyltin was more effective as a fungicide than triethyltin (50) it

seemed much less toxic to mammals (8, 29) but this may only be a reflection of its poor absorption. Tributyltin salts can cause a mild skin burn when splashed during manufacture (62).

Apart from a wave of mitosis in the liver after injection of a fatal dose and some testicular atrophy in long-term feeding experiments the lesions produced by triethyltin compounds were confined to the central nervous system (64). The characteristic lesion was an interstitial oedema extending throughout the white matter of the neuraxis. It was most easily produced by triethyltin but it also occurred after the administration of both tripropyltins and, although less readily, after tributyltin (8, 29). With triethyltin the oedema quickly formed and in feeding experiments it persisted until the rat was restored to a normal diet. The lesion was reversible and was not accompanied by any changes in the appearance of the nerve cells or by degeneration of the nerve tracts, nor was there any oedema in other organs such as the liver. Triethyltin produced this oedema in other mammals including man. This may be concluded from the findings in the 4 fatal cases of "Stalinon" poisoning in which the brain was examined histologically (42). The same distinctive interstitial oedema of the white matter was seen as in animal experiments with triethyltin. While there is every reason to believe that lesions in the victims of "Stalinon" poisoning were due to triethyltin it is not possible to give an accurate estimate of the toxic dose nor to say to what extent the toxic action of diethyltin contributed to the picture. Only two cases received BAL (31), which might have mitigated some of the effects of the diethyltin.

Lower alkyltin compounds may be readily absorbed from the gut but there are species differences in the degree of absorption. For instance, triethyltin was almost non-toxic when given by mouth in fowls although they were very sensitive to triethyltin given parenterally. At the other extreme, in the rat, triethyltin was equally toxic whether given by mouth or intravenously (8).

The distribution of injected triethyltin can be more accurately described as there is a specific method for its estimation (5, 21). The highest concentrations were found in the liver and blood with smaller levels in the kidneys, spleen, heart, brain and skeletal muscle. The same distribution was seen whether triethyltin was given by mouth or by injection and it was not concentrated in the central nervous system. Rats were fed with triethyltin hydroxide so that they consumed 11 mg per rat during 89 days and at the end of that time only 0.7 mg could be recovered; 40% of this amount was in the blood stream, 28% in the liver and 29% in the skeletal muscles. If such rats were returned to a normal diet no triethyltin could be detected in the tissues after 12 days. The exact way in which triethyltin is excreted is not known.

Species variations in the distribution of triethyltin must be borne in mind. When triethyltin was added to rat blood *in vitro* it was taken up by the erythrocytes and none was found in the plasma whereas when added to rabbit blood it became equally distributed between plasma and erythrocytes. These findings probably explain why triethyltin persists in the blood of treated rats whereas it quickly disappears from the blood stream of treated rabbits.

Biochemical action of trialkyltin compounds. Reports on changes in the whole animal are limited to those with triethyltin in the rat. From the point of view of "clinical chemistry" fatal doses of triethyltin caused a moderate hyperglycaemia secondary to the liberation of adrenaline from the adrenal medulla. There was also an increase in the blood non-protein nitrogen which might be secondary to the decreased renal function seen after these doses (97). Urinary porphyrin excretion was strikingly reduced (Prof. C. Rimington, private communication). Triethyltin had no effect on the true or pseudo-cholinesterase of the brain or spinal cord of the rat. *In vitro* triethyltin at a concentration of 1.4×10^{-3} M depressed the pseudo-cholinesterase activity of horse serum 25% (97).

Total O_2 consumption. Triethyltin depressed total O_2 consumption in the rat (96). The threshold dose was about 3 mg triethyltin sulphate per kg body weight. With doses of 6 mg/kg (approx. LD50) and above, the change in O_2 consumption reflected the clinical state of the rat. For instance, after a dose of 12 mg/kg body weight there was an immediate severe fall in O_2 consumption followed by some recovery and then a steady decline until the rat died. Apart from the initial effect the changes were in keeping with the fall in body temperature. Body temperature is rapidly reduced by triethyltin and there is a striking fall in the temperature of both liver and brain (97, 98).

Tissue O_2 tension. The effect of triethyltin on the extracellular O_2 tension in the tissues of the unanaesthetized rat has been determined polarographically (96). The results showed that with doses up to 12 mg/kg the O_2 supply to the tissues was certainly adequate up to the time when the rat was very ill and the rise in tension seen in the early stages in the brain and more constantly in the liver suggested that O_2 utilisation by these tissues was depressed *in vivo*.

Tissue constituents. 1) *Central nervous system.* The most significant change in the composition of the central nervous system of mammals poisoned by certain trialkyltin compounds was the increase in its water content. This increase can be very large and was more marked in the spinal cord than in the brain. The wet weight of the tissue was increased and the increase in size was visible to the naked eye. In young rats the increase in the size of the brain was sufficient to alter the shape of the skull. The anatomical distribution of the excess fluid has been described above. This oedema of the white matter was most easily produced by triethyltin but it also occurred after the administration of both isomers of tripropyltin and after tri-*n*-butyltin if this was given in sufficiently large amounts (8). The oedema was not produced by the higher members of the series nor by trimethyltin. With triethyltin it began to form very soon after the compound was given (98) and, in feeding experiments, persisted for as long as the triethyltin was included in the diet.

The increase in the water content of the central nervous system was accompanied by a large rise in its Na^+ level without change in the K^+ level when expressed on a dry weight basis. The levels of total nucleic acid, total lipid, phospholipid and cholesterol per unit dry weight of brain and spinal cord were also unaltered. It was concluded that the extracellular space was increased without

change in the solids of the central nervous system. Calculations based on the electrolyte results showed that the picture could be explained by distension of the extracellular space of the brain and spinal cord by an ultrafiltrate of plasma (64).

Similarly the only changes in the phosphorus containing components of the central nervous system were those due to the increased water content. There was no reduction in the levels of "energy-rich" phosphates even in moribund rats (98), in spite of the fact that triethyltin is the most effective inhibitor yet described for oxidative phosphorylation in liver and brain mitochondria. This is discussed later in this review.

2) *Liver.* In acute experiments (98) the lipid and residual phosphate fractions of the liver were unchanged after triethyltin but the acid soluble phosphate fraction increased due to a rise in its adenine nucleotide content. The water content of the liver was unaffected.

3) *Muscle.* The phosphate fractions of the muscle were unaltered after fatal doses of triethyltin (97).

Blood-brain barrier. The occurrence of cerebral oedema has led to work on the possible effect of triethyltin on the blood-brain barrier in the rat (64, 98). No abnormal permeability to dyes was discovered and the distribution of sulphonamide between the brain and spinal cord and plasma suggested that its penetration into the spinal cord was reduced in rats fed triethyltin. In acute experiments the penetration of P^{32} into the brain was depressed by fatal doses of triethyltin; the P^{32} was administered as inorganic phosphate.

Effect on incorporation of radioactive phosphate by tissues. This has been studied in the central nervous system and liver of the rat after triethyltin (98). The removal of P^{32} from the plasma was reduced in rats given triethyltin. As pointed out above, its penetration into the brain was lowered but its entry into the liver was unaffected. In the brain its distribution within the acid-soluble phosphate fraction was the same as in the controls except that the relative radioactivity of the diphosphopyridine nucleotide was reduced. There was also a fall in the radioactivity of the lipid and residual phosphate fractions relative to that of the total acid-soluble phosphate. Since there was no hindrance to the passage of P^{32} into the "energy-rich" phosphates this may indicate a reduced utilisation of chemical energy by the central nervous system after triethyltin.

In the liver triethyltin did not affect the distribution of P^{32} in the acid-soluble fraction. The relative labelling of the lipid phosphate was again reduced but the relative specific activity of the residual phosphate fraction was slightly increased. Subsequent work has shown that this is probably due to increased adsorption of P^{32} on to this fraction.

Effect on incorporation of C^{14} labelled amino acids into rat tissue protein. Using the method of Simkin and Work (94) the effect of fatal intraperitoneal doses of triethyltin sulphate (10 mg/kg) on the incorporation of C^{14} labelled amino acids into tissue protein has been studied (Miss J. Cremer, unpublished observations).

Fifteen minutes after the injection of triethyltin C^{14} incorporation was unchanged. Two hours after triethyltin the incorporation into pancreatic protein was lowered 50 % and at 24 hours when incorporation into this tissue was normal

the incorporation into the liver protein was raised 50%. There were no striking changes in the incorporation into the kidney or brain at these intervals after triethyltin. It would be premature to conclude that this was a specific effect of the triethyltin.

Effect on isolated tissues. 1) *Intestine.* The effect of triethyltin on a preparation of isolated rat intestine has been observed (74). It inhibited O_2 consumption and the transport of water and glucose at concentrations of 10^{-6} to 10^{-5} M. Glucose utilisation by the gut was not inhibited until the concentration reached 2×10^{-4} M. On this preparation triethyltin was 10 times more effective than 2,4-dinitrophenol as an inhibitor of transport. Both compounds reduced the adenosine triphosphate content of the preparation but the correlation between the degree of transport inhibition and the fall in adenosine triphosphate level was much closer in the case of 2,4-dinitrophenol. The effect of triethyltin on transport was also observed in the whole animal where it was 100 times less effective than *in vitro*.

2) *Rat phrenic nerve diaphragm.* At 1×10^{-5} M triethyltin caused a slow progressive failure in the response to indirect stimulation complete within 1 hour. A little recovery took place when the triethyltin was removed. Trimethyltin had a similar action but it was readily reversed by washing. This may reflect the relative water/lipoid solubilities of the two compounds. Triethyl and trimethyltin enhanced the action of both (+) D-tubocurarine and decamethonium (97). It was shown that this action was not associated with any decrease in the output of acetylcholine into the bath containing the diaphragm stimulated in the presence of alkyl tin (Mrs. Janet I. Duff, unpublished observation). In the intact animal (rabbit) a single dose of triethyltin impaired the response of the gastrocnemius to a tetanus at 500/sec but not at lower rates of stimulation. This was apparently an acute response to a single dose for the neuromuscular conduction was normal in a rabbit given triethyltin in its diet until a stage of advanced muscle weakness had been reached (97).

3) *Tissue slices.* The metabolism of rat tissue slices after the addition of triethyltin *in vitro* has been studied (21). The respiratory activity of most tissues (liver, kidney, heart, diaphragm, small intestine) was unaffected by low concentrations (3×10^{-7} to 3×10^{-6} M) of triethyltin but the O_2 uptake by slices of brain cortex, brain white matter and spinal cord metabolising glucose was depressed. Much higher (20-fold) concentrations of triethyltin had to be used to produce any effect on other tissues. The effect on the brain cortex slices was not reversed by transferring them to fresh incubation medium. This effect with central nervous system slices is not due to an increased uptake of triethyltin in the tissue for it has been found that all tissues suspended in media take up the material in the same concentrations. Further features of the effect of triethyltin on slices from the central nervous system were an increase in the lactate concentration of the medium with a fall in its pyruvate concentration and an increase in glucose uptake under aerobic conditions. Under anaerobic conditions triethyltin depressed both glucose uptake and lactate formation.

It is of great interest to find the same picture in brain slices prepared from rats injected or fed with triethyltin. Slices prepared from the other tissues of these

rats behaved in the same way as control slices. The concentration of triethyltin in the brain slices from the injected rat was determined and it was shown that a similar metabolic disturbance was produced when triethyltin was added to normal brain slices *in vitro* so as to produce the same concentration in the slice.

Effect on tissue particles. The effect of trialkyltin compounds on the metabolism of tissue particle preparations has been intensively studied (3, 4). The effect of the trialkyl compounds was quite distinct from that of the dialkyl compounds.

Whereas BAL combined with dialkyltin and prevented its biochemical effect, it had no effect on the response to trialkyltin for which it has very little affinity *in vitro*. Glutathione was also without effect.

The effect of triethyltin on brain brei preparations was very similar to its effect on brain slices. With glucose or lactate as substrates 10^{-4} to 10^{-5} M concentrations of triethyltin lowered O_2 uptake and the pyruvate concentration was also lowered. As in the brain slice work, the explanation of this latter finding is not clear but it was shown that it was not due to the inhibition of lactic dehydrogenase.

More information is available concerning the effect of trialkyltin compounds on the metabolic activity of isolated liver mitochondria (3). The mitochondria used in these experiments had a low substrate oxidation rate in an electrolyte medium at 37°C, this rate was capable of being increased 3- to 4-fold by the addition of 2,4-dinitrophenol or of systems utilizing adenosine triphosphate, *i.e.*, by the addition of apyrase, hexokinase and glucose. The mitochondria also showed negligible adenosine triphosphatase activity (Dr. W. N. Aldridge, personal communication). This experimental technique by providing unstimulated and stimulated mitochondria furnished two test systems. In the presence of apyrase or hexokinase and glucose the stimulated oxidation is certainly coupled to phosphorylation.

Members of the trialkyltin series up to tri-*n*-octyltin inhibited both the unstimulated and stimulated oxidation, the latter being the more sensitive. Triethyl, tri-*n*-propyl, tri-*iso*-propyl and tri-*n*-butyltin all inhibited the stimulated oxidation at a concentration of 10^{-6} M. The trimethyl and tri-*n*-hexyltin were less active. The lower members of the series caused considerable stimulation of the unstimulated mitochondrial respiration at concentrations below those causing inhibition. Trimethyl and triethyltin were equally active in this respect but the activity decreased with the higher compounds and tri-*n*-butyltin was almost inactive. These compounds also inhibited oxidative phosphorylation including that associated with the oxidation of succinate, triethyltin being the most active compound. The effect of this compound could be seen at concentrations of 10^{-7} M so that it is the most active inhibitor of oxidative phosphorylation at present known. It was shown that these effects were not due to the loss of essential coenzymes from the mitochondria. It is interesting to compare the activity of the trialkyltin compounds against isolated mitochondria with its toxicity to the whole animal and to fungi. In the whole animal, triethyltin is also the most toxic but there is a progressive falling off in activity with the higher compounds not

seen *in vitro* where there is no serious loss in activity until the tri-*n*-octyltin is reached. The peak of antifungal activity seems to be unrelated to these findings.

By studying the effect of these compounds on the mitochondrial adenosine triphosphatase and particularly under conditions when it has been activated by 2,4-dinitrophenol Aldridge has been able to formulate come conclusions about the mode of action of these compounds on the mitochondria (3).

Members of the series below tri-*n*-octyltin all cause some activation of the latent mitochondrial adenosine triphosphatase but only trimethyl- and triethyltin have a pronounced effect. This action explains the effect of these compounds in increasing the O_2 uptake of the "unstimulated" mitochondrial preparations. The mechanism of this effect is not clear but it is not due to structural damage to the mitochondria. Thus, the most active compound, trimethyltin, produces no mitochondrial swelling at any concentration (Dr. W. N. Aldridge, personal communication). Another type of activation of mitochondrial adenosine triphosphatase is seen at very high concentrations when oxidation is completely inhibited and this is probably due to damage to the mitochondria.

These trialkyltin compounds with the exception of tri-*n*-octyltin also inhibited the activation of mitochondrial adenosine triphosphatase by 2,4-dinitrophenol. In this they differed from respiratory inhibitors such as cyanide, phenylarsenious acid and dialkyltin. The fact that the concentrations required for this effect were the same as those required to inhibit the 2,4-dinitrophenol stimulated oxidation strongly suggests that the point of action of the trialkyltins is the same as that of 2,4-dinitrophenol although the mode of action need not be the same. For this reason it is thought that trialkyltin inhibits a step in the energy-transferring chain between electron transport and the formation of adenosine triphosphate. The results obtained (3) excluded the alternative possibility that the over-all effect was due to the combination of a stimulation of adenosine triphosphatase and inhibition of electron transport.

Although it has been possible to define with some precision the biochemical action of triethyltin on cellular preparations studied in isolation, the information so gained does not make it possible to offer an immediate explanation of the toxic action of triethyltin in the whole animal. Pathological changes are produced in the brain but none is visible in the liver despite the fact that the concentration of triethyltin is higher in the liver than in the brain. A fall in liver temperature does, however, indicate that the triethyltin has had some effect on its metabolism. However, in neither organ is there a fall in the level of energy-rich phosphate compounds such as might be expected after the administration of a substance with such a powerful inhibitory effect upon their synthesis by mitochondrial preparations.

There are no striking differences in the biochemical actions of trimethyl and triethyltin *in vitro* yet in the whole animal differences in behaviour during poisoning are matched by the failure of trimethyltin to produce cerebral oedema although it is almost as toxic as triethyltin. This difficulty of defining exactly the mode of action of a poison in the whole animal is met with in many cases even

where studies undertaken *in vitro* appear to provide a basis for a rational explanation.

CONCLUDING COMMENTS

There is no evidence that tin in its inorganic form can exert any bactericidal action either *in vitro* or *in vivo* but it is more difficult to decide whether or not tin in this form can exert a specific toxic effect on mammals. If large doses of sodium stannous tartrate are injected into rabbits weakness and paralysis can be produced. The lower trialkyltins have powerful antifungal effects, and appear toxic to all forms of life. They are therefore quite unsuitable for therapeutic use. Their inclusion in some preparations of "Stalinon" appears to have been the result of faulty preparation of the diethyltin which was intended to be the active ingredient of this preparation. The dialkyltins are, however, almost inactive as antifungal agents and have no known action against bacteria. The reason for their inclusion in a remedy for bacterial infections has never been disclosed but may presumably stem from the widely held belief that inorganic tin was effective in this respect.

The specific effect of the lower trialkyltins upon the central nervous system is undoubted but whether or not this effect is basically similar to that produced by the stanni-tartrates is uncertain. Alkyl mercury and alkyl lead compounds produce toxic effects in the central nervous system and so do these metals when inhaled as fumes. Not enough is known of the biochemical or pathological features of these toxic actions to make it possible to say whether the damage produced by the metal is the same as that produced by its alkyl derivative.

A clear distinction must be drawn between the effects of the trialkyltin compounds which affect the central nervous system and of the dialkyl compounds which produce lesions elsewhere. On isolated tissues and tissue fragments the action of these two groups is completely distinct. The tetraalkyl tins are inert *in vitro*, but they poison the animal because they become converted to the corresponding trialkyl derivatives. There is, however, no further reaction to produce the dialkyl derivative. Tetraethyllead behaves similarly and the condition produced by triethyllead is very like that produced by trimethyltin (23, 97, 105), and its relative solubilities in fat and water are similar. Nothing is yet known about the biochemical reaction to alkyl mercury compounds, but in man and rat they can produce structural damage to neurones (47).

When present in food, even up to the permitted level, inorganic tin compounds appear to have no potential health hazards. For the alkyltins there are species differences in susceptibility to particular compounds, making prediction from animal experiments uncertain. The higher alkyl homologues—octyl or above—are much less toxic by mouth. The higher dialkyltins can be used because they still function effectively as stabilisers, but as fungicides the trioctyltin salts are ineffective. The tributyltin salts, while much less toxic than triethyltin to laboratory animals, are equally toxic to tissue preparations *in vitro* and are fully effective fungicides. Their use should provide a higher margin of safety than that of the triethyltin to which man is clearly very susceptible. Whether or not their

toxic action on the central nervous system as distinct from that of the alkylmercury compounds provides a balance in favour of the alkyl tins from the point of view of human safety requires further evidence.

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