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SOME INVESTIGATIONS ON THE TOXICOLOGY OF TIN, WITH SPECIAL REFERENCE TO THE METALLIC CONTAMINATION OF CANNED FOODS.

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IN July 1906 attention was drawn to the fact that a large number of tinned foods returned from the South African campaign were exposed for sale on the home markets. The majority of these foods were known to have been from five to seven years in tins, and the possession of this material afforded a rare opportunity for investigating the question of metallic contamination, and for determining how far such contamination was deleterious to the public health.

The following account of investigations into the subject is based on a report to the Local Government Board by Dr G. S. Buchanan and myself (Medical Department: Food Reports, No. 7, 1908) extracts from that report being reproduced with the permission of the Controller of H. M. Stationery Office.

Previous researches dealing with this subject have been published by Ungar and Bodländer (1887), working in Binz's laboratory, and by Lehmann (1902). The former investigators shewed that by repeated subcutaneous injections into animals of small quantities of tin in the form of a non-irritant organic salt (the double tartrate of tin and sodium) over prolonged periods, definite toxic symptoms could be produced, which resulted, after sufficiently long treatment with the metallic salt, in the death of the animal. The general effects of the poison were manifested (*a*) in disturbances in the alimentary tract, (*b*) in the general nutrition, and above all (*c*) in the central nervous system. In the case of rabbits and dogs the disturbances in the alimentary tract were only manifested when relatively large quantities of the salt were injected in the earlier stages of the experiments. In all cases repeated injections caused a marked diminution of weight and general wasting, in spite of the fact that the animals continued to eat well till near the end, especially when the doses of tin salts injected were small. In most cases the wasting was accompanied by anaemia, probably due to the destructive action of the tin salts on the blood corpuscles (Löwenthal, 1902). The most marked changes were produced, however, in the nervous system, and were manifested in the first instance in the general disturbance of the power of motion in the hind limbs resulting finally in complete paralysis with anaesthesia. The animals at the same time exhibited a general depression of higher mental functions and intelligence. The general symptoms described by Ungar and Bodländer indicate not only disturbances of the central nervous system but also peripheral neuritis. They concluded that very small quantities of tin, when absorbed into the system, could cause serious disturbances to health, which could lead finally to a fatal termination. In the case of dogs, a daily subcutaneous dose equivalent to 6·75 mg. of metal per kilo of body weight resulted in the death of the animal after 8 days. With doses equivalent to 1·71 and 1·33 mg. per kilo of body weight, death resulted in 47 and 131 days respectively. These doses would correspond to daily doses of 6·2, 1·57 and 1·2 grains for individuals of 60 kilos weight.

Lehmann continued these investigations with the object of determining whether quantities of tin equivalent to those which could be ingested by the consumption of heavily contaminated canned foods were likely to result in tin poisoning with symptoms like those described by Ungar and Bodländer. He calculated that the maximum amount of tin likely to be ingested by a full grown man of 75 kilos weight, was 420 mg. (about 6·5 grains), the equivalent of 5·6 mg. per kilo. He experimented with cats, giving the animals with their food tin in doses gradually increasing from 5 to 40 mg. daily. The animals after prolonged periods of ingestion remained in perfectly normal health and increased in weight, and Lehmann concluded from the results of his experiments that the possibility of chronic tin poisoning from the consumption of canned foods is extremely remote.

Apart from the question of chronic poisoning, it is necessary to consider the irritant action on the alimentary tract which can be produced by the ingestion of relatively large quantities of tin salts, especially when the latter are, as tin chloride is, of somewhat corrosive nature. Records of severe disturbances to the alimentary tract following the ingestion of canned foods are to be found in the literature, of which the best authenticated cases are described by Ungar and Bodländer (1887), Luff and Metcalfe (1890) and Günther (1899). In all these cases estimations of the metallic contaminations of the food-stuffs were made. Ungar and Bodländer found that various tins of the canned asparagus to which the illness was ascribed contained from 2·1 to 2·8 grains per pound (300 to 400 mg. per kilo). In Luff and Metcalfe's cases, canned cherries contained no less than 24 grains to the pound (3430 mg. per kilo), whereas in Günther's case pickled herrings preserved in vinegar contained 7·2 grains per pound (1030 mg. per kilo) in the solid, and 2·2 grains per pound (316 mg. per kilo) in the liquid contents of the tin. The general results of previous investigators tend to shew therefore, that whilst chronic tin poisoning is unlikely to result from ingestion of canned foods, the possibility of irritant action cannot be entirely excluded. These conclusions are in the main confirmed in the present inquiry, where direct experimentation has been possible.

The detection and estimation of tin in food-stuffs and tissues.

Investigations were carried out as to the best methods for determining the amount of tin in food-stuffs and tissues and two were finally adopted, viz. a colorimetric method, specially adapted for estimation of small quantities, and a gravimetric method, which was chiefly employed when relatively large quantities of the metal were present.

Colorimetric method. The reagent used for the colorimetric determination was dinitrodiphenylaminesulphoxide

$$NH\begin{matrix} \diagup C_6H_3(NO_2) \diagdown \\ \diagdown C_6H_3(NO_2) \diagup \end{matrix}SO$$

which was originally prepared by Bernthsen (*Liebigs Annalen,* 1885, CCXXX. 116) by the action of nitric acid on thiodiphenylamine.

This substance on treatment with stannous salts is reduced to the corresponding diamino-derivative, which is the leuko-base of a dye-stuff, known from the name of its original discoverer as Lauth's violet. For the purposes of the analyses, 10 grams of the food-stuff or tissue are introduced into a 700 c.c. round-bottomed Jena flask together with about 10 grams of potassium sulphate; 10 c.c. of concentrated sulphuric acid are then introduced together with some water. The whole is then gently heated, and the diluted sulphuric acid first hydrolyses the proteins into simpler substances, which are more readily destroyed by the acid than the original protein. After the mixture has charred and frothed up, more sulphuric acid is added, and the heating is continued till the whole of the organic matter has been destroyed. The amount of acid required for destruction will depend upon the nature of the tissue or food-stuff, and the amount of water it contains; 20 c.c. were in most cases sufficient, but in exceptional cases such as those of jams and meat extracts, containing large quantities of solid matter, as much as 40 c.c. were found necessary. After destruction of organic matter and cooling the contents of the Kjeldahl flask were diluted to 100 c.c., the liquid was saturated with sulphuretted hydrogen, and allowed to stand in a corked flask over night. The precipitated sulphur and sulphides were then filtered off on to a small filter paper (4 cm. diameter) and washed. The paper containing the precipitate was then transferred to a test-tube and boiled with 5 c.c. concentrated hydrochloric acid. After solution of the sulphide, the liquid was filtered through a Buchner funnel by means of a pump into a wide-mouthed test-tube with side-tube near the top by means of which it was connected with the pump. The filter paper remaining on the funnel was sucked as dry as possible, and washed with 2½ c.c. of strong hydrochloric acid, the washings being added to the filtrate. The test-tube was then connected with an apparatus for generating carbon dioxide, and the gas was led by a tube passing through a bored cork in the test-tube just over the surface of the liquid, the side-tube serving for its exit. A strip of zinc foil 2 inches long and ½ inch wide, and weighing about ¾ gram was introduced into the liquid while still hot, and the stannic chloride was thereby reduced to stannous salt in the presence of a current of carbonic acid gas. As soon as the *last traces* of the zinc had dissolved, the reagent was added. This was made by dissolving 0·2 gram of dinitrodiphenylaminesulphoxide in 100 c.c. of $\frac{N}{10}$ sodium hydroxide and filtering. 2 c.c. were employed for each test, the cork being momentarily removed from the test-tube for its introduction, and the carbon dioxide being continually passed the whole time. The nitro-body was precipitated at first, but redissolved on further warming. After heating for one or two minutes the mixture was diluted to 100 c.c. with cold water, one or two drops of ferric chloride solution were added, and the mixture was filtered. In the presence of tin a violet colour appeared, the intensity of which served as a measure of the amount of tin present in the substance analysed. The whole process, after the filtration of the sulphide, only takes a few minutes, and as a rule several analyses were carried out simultaneously. For determining colorimetrically the amount of tin, a standard solution containing 14·28 mg. in each 5 c.c. was prepared. This was diluted with 5 c.c. of water, and 1 c.c. of the diluted solution contained therefore 1·428 mg. This quantity added to 10 grams of food-stuff was equivalent to a contamination of 1 grain per pound. For preparation of standard colours, 1 c.c., 0·75 c.c., 0·5 c.c. and 0·25 c.c. respectively, of the diluted solution were added to 7·5 c.c. of concentrated acid. Zinc (of standard size as above) was added, and the stannic salt reduced to stannous salt in a current of carbon dioxide and the reaction carried out with the dinitrodiphenylaminesulphoxide reagent in the way already described. A series of standard colours could thus be prepared, to serve as a comparison for those produced by the tin-contaminated food-stuffs or tissues. It must be remembered that the depth of colour is not in proportion to the amount of tin; the reaction is less complete in the more dilute experiments and the influence of mass action must be taken into account. The colour given by quantities of tin equivalent to 1 grain per pound (1 : 7000) is deep purple, and it is necessary to carry out the determination with diluted aliquot parts of the original solution when the contamination exceeds this amount. The colour is quite marked with quantities equivalent to ¼ grain per pound.

Gravimetric estimation. For the purposes of gravimetric estimation, the organic matter was destroyed by heating with a mixture of sulphuric acid and potassium sulphate. After solution, the tin was precipitated by sulphuretted hydrogen as sulphide, which was filtered off, washed, oxidised in the usual way, and finally weighed as SnO_2. With samples containing more than 1 grain of tin, 50 grams of material were sufficient for each analysis, for those containing smaller quantities the colorimetric method was preferable. The latter was, however, always employed as a matter of routine for sorting purposes.

The contamination of foods by tin.

A large number of estimations of tin in different kinds of food-stuffs were carried out, of which the full details are given in the original report. The flesh foods from South Africa, of the approximate age of 6—8 years, contained quantities of tin varying from 0·36 to 2·06 grain per pound (51 to 294 mg. per kilo) the higher quantity having been found in a sample of tripe. The majority did not contain, however, more than 1 grain per pound. Meat essences, extracts and soups contained, however, larger quantities, the contamination being generally more than 1·5 grains per pound, reaching in one case to more than 5 grains per pound. In several cases the contamination was 3·5 grains per pound, and in one tin of meat essences into which the solder had dropped the contents contained more than 21 grains per pound. Puddings and jams were also somewhat heavily contaminated, the quantities of tin ranging from 1·42 up to 5·13 grains per pound (203 to 733 mg. per kilo). It is conceivable therefore that in certain cases injurious results can accrue from ingestion of old canned foods. In addition to analyses carried out for the purposes of the report, data referring to tin contamination of foods were found in the literature and a numerical summary of the results obtained by different observers and by myself is given in the accompanying table. The summary has been made as a convenient way of exhibiting the results of some 130 analyses of different canned foods (exclusive of cans which had been punctured, or those in which obvious contamination by solder had taken place). It should be remembered, however, that although most of the foods in question have been "old," the periods of keeping are very varied and the results have been obtained by different methods.

	No.	Percentage of total
Food-stuffs containing less than 1 grain per pound	72	55·4
,, ,, between 1 and 2 grains per pound	35	26·9
,, ,, ,, 2 and 3 ,, ,,	17	13·0
,, ,, more than 3 ,, ,,	6	4·6

Physiological experiments.

The animal experiments of Lehmann (1902) render it improbable that tin, when ingested in quantities such as are found in contaminated canned foods, can cause symptoms of chronic poisoning. The experiments recorded in this section tend to confirm Lehmann's conclusions, and to shew that by far the largest quantity of the metal ingested *per os* is excreted without leaving the alimentary tract.

The first experiment was carried out on the person of the author of this communication, who at the time was in normal health and weighed 65 kilos. The metal was ingested in the form of the non-irritant double tartrate of tin and sodium, which was taken continually for a period of three weeks. During the first week the daily quantity ingested was approximately 1 grain (64·5 mg.), during the second week two grains (129 mg.) and during the third week 3 grains (193·5 mg.). The intake commenced on a Saturday at mid-day meal and was continued throughout the week till breakfast on the following Saturday. Four meals were taken each day and a quarter of the daily dose was taken at each meal. This during the first week amounted to $\frac{1}{4}$ grain. On Saturday at luncheon after the first week the dose was increased to $\frac{1}{2}$ grain, and on the following Saturday at the corresponding meal to $\frac{3}{4}$ grain. From Monday morning at 9.30 to the corresponding time on the following Saturday in each week, the total excreta, both urine and faeces, were collected; the quantities collected in each 24 hours were kept separately and separately analysed. No standard diet was adopted, although an effort was made to live as regularly as possible. In the faeces the total nitrogen and tin were estimated and in the urine the total nitrogen, the ammonia, the urea, uric acid, and the tin.

The output of tin during three successive periods of five days was thus determined and compared with the intake. It must be remembered that the main object of this experiment was to determine whether the excretion of tin kept pace with the intake, and for this reason, the method of analysing the excretions during a definite period in each week was purposely chosen. The method is, however, not without sources of error, the chief of which is due to the fact that it is impossible to get perfectly regular defaecation, in which case the faeces excreted during a certain period would not correspond with the material ingested. It was thought, however, that with periods as long as five days, this source of error would not be very great. The total nitrogen of the faeces and urine were, however, both determined, and it was found that the ratio of the nitrogen excreted in the urine to that excreted in the faeces did not vary greatly from week to week, and these small variations, furthermore, bore no relation to the proportion of tin in the excretions. Some of the chief results are indicated in the following table.

Urine Analyses.

	Total nitrogen excreted (grams)	Total tin excreted (grams)	Total tin ingested during 5 days (grams)
Five days of first week April 23rd—27th	76·9	0	·3300
Five days of second week April 30th—May 4th	72·2	·0550	·6600
Five days of third week May 7th—May 11th	70·8	·0763	·9900

The analyses of urea, ammonia, etc., indicated that no disturbances of metabolism had occurred during the period of tin ingestion.

Analyses of Faeces.

Date	Weight of faeces excreted (grams)	Per cent. N.	Total N. (grams)	Per cent. Sn.	Total Sn. (grams)	Tin Ingested
April 23	95	1·31	1·24	·0383	·0364	Approximately 1 grain per day.
,, 24	121	1·68	2·03	·0693	·0838	
,, 25	150·5	1·36	2·04	·0511	·0769	
,, 26	58·7	1·90	1·11	·0746	·0438	
,, 27	139	1·84	2·55	·0733	·1019	
Total 5 days	564·2	1·59	8·97	·0607	·3428	·3300
April 30	132	1·91	2·52	·1123	·1482	Approximately 2 grains per day.
May 1	69	1·96	1·35	·1397	·0964	
,, 2	91	1·92	1·73	·1251	·1138	
,, 3	73·5	1·95	1·41	·1209	·0888	
,, 4	156	1·67	2·60	·1209	·1885	
Total 5 days	521·5	1·84	9·61	·1218	·6357	·6600
May 7	89	1·36	1·21	·1470	·1308	Approximately 3 grains per day.
,, 8	141	1·69	2·38	·1740	·2453	
,, 9	112	1·73	1·93	·1354	·1516	
,, 10	52	1·80	0·94	·1846	·0966	
,, 11	105	1·87	1·96	·1797	·1886	
Total 5 days	499	1·69	8·42	·1628	·8123	·9900

The following table gives the ratio between the nitrogen excreted in the urine and faeces.

	Total Nitrogen ingested calculated from N. in urine and N. in faeces (grams)	N. in urine (grams)	N. in faeces (grams)	Physiological food value	Percentage unutilized N.
First week	85·9	76·9	9·0	89·5	10·5
Second week	81·8	72·2	9·6	88·3	11·7
Third week	79·2	70·8	8·4	89·4	10·6

The following table summarises the results and indicates the paths of excretion of the metal.

	Tin ingested (grams)	Total tin excreted (grams)	Total in urine	Total in faeces	Percentage of total tin recovered which was Present in urine Per cent.	Percentage of total tin recovered which was Present in faeces Per cent.
First week	·3300	·3428	·0	·3428	·0	100
Second week	·6600	·6907	·0550	·6357	7·9	92·1
Third week	·9900	·8886	·0763	·8123	8·6	91·4

From the above results it is evident, that when the quantities of tin ingested were one or two grains per day, no evidence of accumulation was forthcoming at the end of a fortnight. The quantities excreted are substantially the same as those ingested, the differences lying well within the limits of experimental error. In the first week the quantities of tin excreted in the urine were so minute, that it is difficult to state with certainty that even traces were present. In the second week there was distinct evidence that some of the metal had been absorbed from the alimentary tract, and the amount excreted in the urine amounted to 8 per cent. of the total. When the quantity of tin ingested reached the amount of 3 grains daily, however, the output failed to keep pace with the intake, the deficiency in the excretion amounting to 10·3 per cent., a larger quantity than could be accounted for by any irregularity in the defaecation, as reference to the table shews that the ratio of nitrogen excreted in the faeces to the total nitrogen ingested was the same as that in the first week, when the tin ingested was quantitatively recovered in the faeces.

Similar experiments were carried out with a dog weighing 6·7 kilos, which received with its food during the first week 10 mg. daily, during the second week 20 mg. daily and during the third week 30 mg. daily. Practically the whole of the tin was recovered in the faeces.

The above experiments indicate at first sight, that of tin ingested *per os* comparatively little leaves the alimentary tract. Ungar and Bodländer have shewn, however, that when tin is subcutaneously injected, part leaves the system in the faeces. It is therefore possible that some of the tin excreted in the faeces in my experiment, had been absorbed before excretion into the gut. The following experiments indicate, however, that when the metal has been once absorbed into the system, the great part leaves it again in the urine, and that, therefore, there is but small absorption from the alimentary tract.

A medium-sized dog (about 7 kilos) received daily a subcutaneous injection of five milligrams of tin in the form of a double tartrate for six days. 127 grams of faeces (dried weight) were collected and also the whole of the urine excreted during the period of experiment and the three subsequent days. A quarter of the total of excreta were submitted to colorimetric analysis for tin, and it was found that a quarter of the urine contained about 1·5 mg. of the metal, *i.e.* 6 mg. of the total of 30 mg. were found in the urine. The faeces contained only about two-thirds of this quantity.

A second experiment with another animal, which received 20 mg. of metal subcutaneotısly, lead to a similar result. In both these cases small doses of the metal only were injected. In the following experiment relatively large quantities of tin were injected within a short interval. In this case, a considerable proportion is rapidly excreted by the urine.

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A dog of 8·5 kilos weight, one hour and a half after an injection of morphia, was fully anaesthetised with A.C.E. mixture. Cannulae were then inserted in Wharton's duct and the ureters, and a manometer was connected with the femoral artery. 100 milligrams of tin in the form of the double tartrate dissolved in 100 c.c. of warm physiological saline were then injected into the external jugular vein. No change in the blood pressure was caused thereby. During the course of the next hour and a half, 34 c.c. of urine were obtained and 10 c.c. of saliva, the latter being produced by electrical stimulation from time to time of the corda tympani. Throughout the whole experiment the animal was kept in a state of complete anaethesia, and it was killed by a heart incision whilst still under the influence of the anaesthetic. The saliva, urine, and various organs of the body were then submitted to colorimetric analysis for the purpose of estimating approximately the distribution of tin.

The mucous membrane of the large intestine weighed 8 grams, of which 4 grams were used for analysis. Tin was found to be absent.

The mucous membrane of the small intestine weighed 93 grams, of which 25 grams were used for analysis. In this quantity were found about 0·5 mg. Sn.

The liver weighed 218 grams, of which 25 grams were used for analysis. In this quantity were found about 2 mg. Sn, indicating therefore a very appreciable amount in the whole of the liver.

The central nervous system (brain and spinal cord together) weighed 65 grams, of which 20 grams were used for analysis. This quantity was found to contain 1·5 mg. Sn.

Tin was absent from the saliva.

34 c.c. of urine were found to contain no less than 18·4 mg. Sn, which was estimated gravimetrically in 17 c.c. of the liquid.

Conclusions.

The general content of the above work may be briefly summarised as follows:

1. A method is described by means of which small quantities of tin in food-stuffs and tissues can be rapidly estimated colorimetrically.

2. A summary is given of a large number of analyses of canned foods contaminated by metal, most of which had been in tins for several years.

3. As a result of the experiment by Lehmann on animals, and an

experiment described above on a human being, it is concluded that there is but little likelihood of chronic tin poisoning resulting from ingestion of canned foods.

4. Nevertheless, cases have been recorded in the literature, describing symptoms of irritant poisoning following the ingestion of contaminated foods, and in certain of these cases the amount of metallic contamination has been ascertained.

5. It is difficult to state the exact quantity of tin salts which will give rise to symptoms of irritant poisoning, and these toxic effects will vary greatly with circumstances. Quantities of tin approximating to two grains to the pound are, however, unusual and unnecessary, and any food-stuffs containing such quantities should be regarded with suspicion.

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