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PHYSICAL STABILITY AND TINPLATE DETERIORATION IN CANS OF EVAPORATED MILK HELD UNDER REFRIGERATION

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ABSTRACT

Average changes after 4 yr in tin and iron content were not significant in comparison with the fresh control. However, there was a considerable can-to-can variation. The lead content was not significantly changed in storage. The visual color was not significantly darker, although reflectance at 400 mμ did indicate formation of browning compounds. The flavor (hedonic scale) was significantly changed in storage, when consumed as a beverage, but not in coffee. Milk fat separation, protein sedimentation, and line grain (mineral deposit) were found not to be problems under these storage conditions. The pH dropped slightly (0.1 pH), whereas viscosity and whippability were normal and appeared unchanged relative to fresh controls. Vitamins B_1 and B_2 did not change, but B_6 was reduced 25% and B_{12} reduced 33% of the original values. Rat feeding tests did not indicate a significant difference in protein quality between storage and control samples. There was some evidence of an increase in NPN and a substantial increase in free fatty acids during storage. Electrophoretic patterns were essentially the same for both the storage samples and controls.

In May of 1955 a quantity of evaporated milk was purchased by the Quartermaster Corps and stored at 32 F. In the summer of 1959 representative samples from each lot were supplied the Evaporated Milk Association, with the understanding that members of its Administrative Technologists Committee (later to become the Research and Development Council) would subject this milk to physicochemical analyses. The objective was to determine the degree of change that had taken place in the five-storage period during which time the cans of evaporated milk were not turned or disturbed by moving. The balance of the evaporated milk was supplied to range and was accepted without comment.

EXPERIMENTAL PROCEDURE

Iron, tin, and lead. The samples tested for iron were wet-ashed prior to analyses, using redistilled reagents checked by blank determinations. Iron was determined colorimetrically (7) by a thiocyanate procedure, using isoamyl alcohol to extract the color complex. Tin and lead were determined (1) on a Sargent Recording Polarograph (Model XXI). Tin and lead were removed from the acid digest by coprecipitation with added aluminum reagents.

Color. For color measurements a Beckman Model DU Spectrophotometer with reflectance attachment was used at wave lengths of 520 mμ (5) and 400 mμ (3), with slit widths of 0.7 mm and 0.025 mm, respectively. A block of USP carbonate of magnesium was used as a standard to equal 100% reflectance.

Whipping. Whipping quality values were obtained as follows: Milk samples, mixing bowl and beaters from conventional home-kitchen equipment were cooled to 35–42 F; 200 g of milk were poured into the cold mixing bowl and whipped 2 min. The resulting product was poured into a [illegible] graduated cylinder (first precooled) and the volume of whipped milk was

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read immediately and recorded as per cent overrun. The graduated cylinder was left to stand at room temperature. At intervals of 30, 60, and 90 min, the breakdown of the whip was observed by noting the amount of serum drainage.

Organoleptic examination. The flavor of the 4-yr-old evaporated milk samples and the fresh control was evaluated as a beverage after reconstitution to a ratio of one part evaporated milk to one and one-fourth parts water and the evaporated milk was used directly to cream coffee. Forty judges evaluated each sample under these conditions, using the hedonic scorecard—a nine-point scale ranging from extreme like (rated nine) to extreme dislike (rated one) with neutral like or dislike receiving a value of five.

Creaming and protein sedimentation. The amount of creaming (fat separation) and protein sedimentation (sludging) was rated subjectively. The minimal amounts of sediment and fat separation observed would not justify the efforts of quantitative estimation. On a relative basis, however, ratings obtained could be considered on a scale of intensity where a 2-yr-old room temperature sample was graded definite for fat separation.

Viscosity, pH, and lime grain. The viscosity was measured by both the Mojonnier and the Brookfield viscosimeters at 75 F. The pH was measured with the Beckman Model G Glass Electrode. Lime grain measurements were rated subjectively. This term is used to describe a calcium phosphate–citrate complex which forms hard, white, spherical crystals during storage.

B Complex vitamins. Riboflavin and thiamine were determined by fluorometric methods. Vitamin B_6 was determined microbiologically using the yeast, *S. carlsbergensis*, by comparison with fresh USP standard yeast cake. The USP method (Fourth Supplement, XIV, Revision 14, 1950) was used in determination of vitamin B_{12}.

Protein quality. The study of protein quality was made using Sprague-Dawley rats and was continued for 6 wk. Seven groups of ten rats were used. Food was supplied ad libitum, and daily records on the food consumption were kept. Protein was supplied at a level of 9% on a dry basis. The rations were balanced between groups on a caloric basis and they were adequately supplemented with thiamine, riboflavin, vitamin B_6, calcium pantothenate, niacin, vitamin B_{12}, and minerals.

Free fat acidity content. Free fat acidity content was determined by the method described by Frankel and Tarassuk (4). The units of free fat acidity were computed as the milliliters of normal alkali required to neutralize the fat from the ether extract of evaporated milk calculated at the 100-g fat level.

Nonprotein nitrogen. The nonprotein nitrogen was determined by Rowland's procedure. Reagents of the same concentration described by Rowland were used; however, the volume of reagents and the sample size were reduced proportionately (6).

Electrophoresis determination. The electrophoresis was made in an Aminco Electrophoresis apparatus. The protein fraction of the evaporated milks was prepared for electrophoresis as follows: Three parts of veronal buffer, pH 8.4, were added to one part (by weight) of evaporated milk. To 50 ml of this solution 2 ml of M/2 $K_2C_2O_4$ were added with stirring. The solution was centrifuged at 26,000 × g for 20 min to remove precipitated CaC_2O_4 and milk fat. The clarified solution was dialyzed against two changes of veronal buffer at 2 C, to remove lactose and salts. A third dialysis of 18-24 hr with stirring was made against buffer subsequently used in the electrophoresis. The conductivity of the protein solution at electrophoresis did not differ greatly from that of the buffer. The average time run was 2.7 hr.

RESULTS

Iron, tin, and lead analyses. The data for iron content (Table 1), when analyzed for variation among lots and between cans within lots, revealed no significant differences among production lots, but significant differences were observed between duplicate cans. The same type of variation was observed in the fresh controls. The average for the 4-yr-old samples was not significantly different from the fresh control—1.8 vs. 2.0 ppm.

The analysis for tin (Table 1) revealed some pronounced differences, both among the various lots and between duplicate containers within lots. This might suggest that the degree of etching of the tin plate of the can interior would be a good indication of the amount of tin to be found in the milk. However, in this study the correlation was poor. With respect to the increase in tin content over the control, we note significant increases in some cans and lots, although others did not show significant increases. Results for lead content (Table 1) showed no significant differences between the storage samples and the fresh controls. The actual level observed was at the lower limits of sensitivity of the method. These values are consistent with values reported for fresh milk (2).

TABLE 1
Analyses of refrigerated stored QM evaporated milk samples

Sample	Iron parts per million		Mean	Tin parts per million		Mean	Lead (duplicate) parts per million	Etching
7621	1.7	1.6	1.6	5.7	5.1	5.4	Less than 0.1	Moderate—uniform
7621	1.6	1.8	1.7	1.1	1.7	1.4	Less than 0.1	Moderate—uniform
Mean			1.7			3.4		
7622	1.6	2.2	1.9	1.0	1.5	1.7	Less than 0.1	Strong moderate—uniform
7622	1.6	1.5	1.5	1.1	1.1	1.1	Less than 0.1	Strong moderate—uniform
Mean			1.7			1.4		
7623	1.5	1.2	1.3	0.7	1.1	0.9	Less than 0.1	Moderate—uniform
7623	1.8	2.5	2.1	1.7	1.0	1.3	Less than 0.1	Trace general—severe localized
Mean			1.7			1.1		
7689	1.0	1.4	1.2	1.5	1.3	1.4	Less than 0.1	Strong moderate—uniform
7689	2.4	2.3	2.3	1.3	1.1	1.2	Less than 0.1	Slight body—severe localized
Mean			1.8			1.3		
7694	1.2	1.2	1.2	9.5	8.6	9.05	Less than 0.1	Strong moderate—uniform
7694	1.2	1.1	1.1	7.1	7.0	7.35	Less than 0.1	Strong moderate—not quite uniform
Mean			1.2			8.20		
7695	1.4	1.2	1.3	0.2	0.2	0.2	Less than 0.1	Moderate—uniform
7695	2.9	2.7	2.8	0.9	1.1	1.0	Less than 0.1	Strong sl.—uniform
Mean			2.0			.6		
7696	0.9	1.0	0.9	1.9	2.2	2.0	Less than 0.1	Strong moderate—uniform
7696	5.0	4.8	4.9	6.8	6.3	6.5	Less than 0.1	Severe along seams—balance trace
Mean			2.9			4.3		
7699	1.2	1.1	1.1	1.9	2.1	2.0	Less than 0.1	Severe—uniform
7699	1.1	1.3	1.2	2.1	1.9	2.0	Less than 0.1	Severe—uniform
Mean			1.2			2.0		
General mean			1.8			2.79		
Fresh controls								
GT070H	2.4	2.6	2.5	1.3	1.0	1.2	Less than 0.1	Trace—uniform
GT6703	1.3	1.4	1.3	1.8	1.8	1.8	Less than 0.1	Trace—uniform
Mean			1.9			1.5		

Color. The reflectance values shown in Table 2 show a small, but significant, decrease compared to the fresh control (65.7% vs. 68.0% at 520 mμ). This difference should be barely distinguishable by visual comparison. Visual sensitivity to reflectance differences is approximately 1.5% at this wave length.

At 400 mμ the average reflectance value for these stored samples was 46.5%, against a control of 55.5%. This would indicate the development of components capable of absorbing the lower wave length to a greater extent than with fresh samples without necessarily affecting visual color.

Whippability. Data shown in Table 3 indicate the magnitude of variation in whipping quality observed in successive production lots of evaporated milk. There is no basis to assume that there has been any significant change in whipping quality as compared to the fresh control.

Organoleptic evaluations. The mean hedonic values for the flavor comparison of the fresh and the aged evaporated milk when used in coffee and as a beverage were determined. No significant difference was observed when tested in coffee. The value for the storage product was 6.0 in coffee, compared to the control value

TABLE 2

Per cent color reflectance

Slit opening / wave length	0.7 mm 520 mμ	0.025 mm 400 mμ
(Lot no.)	(%)	
7621	65.0	45.5
7622	65.5	46.5
7623	66.8	43.5
7689	66.0	49.0
7694	64.5	46.3
7695	65.0	47.2
7696	66.0	47.5
7699	66.5	46.5
Mean	65.7	46.5
Fresh control	63.0	55.5

TABLE 3

Whipping quality

	% Serum drainage			
	Overrun	30 Min	60 Min	90 Min
(Lot no.)	(%)			
7621	390	5	25	65
7622	350	5	30	70
7623	390	5	50	80
7689	365	5	15	65
7694	320	2	10	17
7695	385	5	35	70
7696	395	32	65	92
7699	400	10	50	85
Mean	374.4	8.6	35.0	68.0
Fresh control	370	10	45	65

of 5.8. However, there was a reduction in score when used as a beverage. The storage samples scored 5.3 as a beverage, compared to 6.1 for the fresh control.

Creaming and protein sedimentation. Observations were made on creaming (fat separation) and protein sedimentation (sludging), comparing the samples with a commercially produced evaporated milk held 2 yr at room temperature. Only slight changes were observed. The minimal amounts of creaming and protein sedimentation that occurred would suggest no necessity for turning evaporated milk, even upon extended storage when the temperature is at 32 F.

Viscosity, pH, and lime grain. These data, shown in Table 4, indicate the viscosity to be consistent with the fresh control. The pH had dropped slightly relative to the control. The lime grain ratings were somewhat variable, but were well within acceptable limits (trace to light).

B Complex vitamins. Results for vitamins B_1, B_2, B_6, and B_{12} are shown in Table 5. The storage samples can be compared with fresh production from the same source. In Table 5 we see no significant difference in vitamins B_6 or B_{12}. Vitamin B_2 is down to a value three-fourths of the original and B_1 to a value two-thirds of the original. More evidence of the stability of thiamine can be seen by comparing the results for B_1 from Table 5 with control samples from the same production area taken a year after production of the storage samples and analyzed soon after processing. The control group showed a mean of 0.54 mg per liter, with a range of 0.46 to 0.64 mg per liter, based on ten individual samples. It was noted there was no significant difference between the two groups.

Similar data were obtained for riboflavin. Two separate series of analyses—one in 1956 and a second in 1960—show the consistency of vitamin B_2 over a 4-yr span. The 1956 samples show an average of 3.91 mg per liter based on ten individual samples. The 1960 samples show an average of 2.99 mg per liter for the eight samples analyzed. When we compare these analyses on fresh product with the values for the cold storage samples, we note a loss of about 25% on the average.

TABLE 4

Evaluation of 4-yr-old evaporated milk

Code	Viscosity M.U.	Viscosity c.p.	Viscosity Temp F	pH	Lime grain
7621	14	21.9	74	6.18	Trace
7622	14.7	26.2	75	6.18	Light
7623	15.7	25.2	75	6.17	Light
7689	16.3	23.2	74	6.18	Trace
7694	28.3	56.2	74	6.15	Light
7695	21	31.5	73	6.13	Light
7696	13.7	22.3	75	6.20	Light
7699	14	21.2	75	6.18	Light
Mean	17.1	28.7	74.4	6.17	
Fresh control	16.7	20.3	75	6.29	None

Protein quality. Table 6 shows the average gain rate over the 6-wk feeding period, and the average gain per gram of protein for six lots of cold storage evaporated milk and one fresh control. The mean gain for the six lots is almost identical to the control—2.84 vs. 2.91 g gain/gram protein. Thus, it appears that by rat assay there has been no significant loss in protein quality by storing at 32 F through 4 yr.

Nonprotein nitrogen, free fatty acids, and electrophoretic mobility. Data covering the above characteristics are in Table 7. First, we note one particular lot (7623) to be uniquely high in NPN—0.107 vs. 0.086% for the mean. The mean value is about 15% higher than the control. The free fatty acid content was somewhat more variable than NPN among the storage samples. Lot 7689 was especially high—1.052 vs. 0.579% of the fat for the mean. The mean value for the aged production samples was 50% higher than the control, which indicates some lipid hydrolysis is taking place even at 32 F.

The electrophoretic patterns were essentially the same for both storage samples and the control.

TABLE 5

Vitamin content of evaporated milk
Stored 4 yr at 32 F

Sample	Thiamine	Riboflavin	Vitamin B_6	Vitamin B_{12}
	(mg/liter)			(μg/liter)
7621	0.54	3.20	0.55	1.7
7622	.55	2.99	.52	1.6
7623	.51	3.08	.42	1.5
7689	.55	3.21	.43	1.5
7694	.60	2.91	.51	1.8
7695	.51	2.82	.49	2.0
7696	.62	2.90	.42	1.9
7699	.58	2.86	.43	1.8
Mean	.56	2.99	.47	1.73
Control G23	.53	3.84	.47	....
G2	.62	3.84	.42	....
G7	.61	3.74	....	....
GT070 B	....	.....	....	2.4

TABLE 6

Protein quality and basic composition
4 yr—32 F evaporated milk

Sample	Protein	Fat	Total solids	Avg 6 wk rat gain	Avg gain
	(%)			(g)	(g/g protein)
7621	7.50	7.93	26.65	114.7	2.84
7623	7.30	7.91	26.21	111.4	2.92
7694	7.57	7.90	26.81	108.3	2.75
7695	7.44	7.89	26.36	110.1	2.83
7696	7.32	7.92	26.10	112.8	2.84
7699	7.31	7.91	26.26	110.3	2.83
Mean	7.41	7.91	26.41	111.3	2.84
Control G23	7.22	7.95	26.11	116.8	2.91

TABLE 7

Electrophoretic mobility

Lot	Nonprotein nitrogen	Free fatty acid	Descending components I	Descending components II	Ascending components I	Ascending components II
	(%)		(cm^2 $volts^{-1}$ sec^{-1})			
7621	0.083	0.444	5.1	2.6	7.8	5.1
7622	.082	.512	5.3	2.6	7.8	5.0
7623	.107	.568	5.0	2.5	7.6	4.5
7689	.081	1.052	4.7	2.3	7.9	4.9
7694	.084	0.580	5.0	3.4	7.5	5.3
7695	.081	.500	4.7	2.9	7.5	5.3
7696	.082	.396	4.9	2.9	7.9	5.0
Avg	.086	.579				
Fresh control	0.074	0.380	5.4×10^{-5}	2.7×10^{-5}	6.9×10^{-5}	4.4×10^{-5}

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A Method for the Determination of Tin in the Range 0·2 to 1·6 μg, and its Application to the Determination of Organotin Stabiliser in Certain Foodstuffs

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Tin is determined spectrophotometrically as its coloured complex with catechol violet, the complex being separated from excess of catechol violet by chromatography on asbestos or cellulose.

Organotin stabiliser is isolated from the foodstuff, separated from inorganic tin by paper chromatography, wet oxidised with sulphuric acid - hydrogen peroxide mixture, and tin determined in the resulting solution.

Organotin compounds used to stabilise poly(vinyl chloride) during container-forming operations can migrate into foodstuffs packaged in such containers. The American Food and Drugs Administration Regulations permit the presence in certain foodstuffs, as a result of such migration, of two organotin compounds, namely, di-octyltin *SS'*-bis-(iso-octyl)mercaptoacetate) and di-octyltin maleate polymer. The concentration of either, or any combination of both, may not exceed 1 p.p.m., which represents 0·158 or 0·259 p.p.m. of tin (as organotin), respectively, in the foodstuff. Convenience and necessity may limit the amount of foodstuffs available for analysis to, say, 5 g. In such a situation a procedure for the determination of about 0·75 μg of tin is essential.

The Analytical Methods Committee's Recommended Method[1] for the determination in organic matter of amounts of tin not greater than 30 μg involves a spectrophotometric finish with the coloured complex of tin with catechol violet. This method was the starting point of attempts to develop a suitable procedure.

Experimental

Determination of Tin—

The tin - catechol violet complex formed from less than 1 μg of tin, under the conditions of the Analytical Methods Committee's procedure, cannot be measured spectrophotometrically in the presence of excess of reagent.

The complex thus formed can be retained on a column of asbestos (some batches of cellulose powder are also suitable) prepared in sodium acetate - hydrochloric acid buffer solution of pH 3·8. Excess of catechol violet is not retained, and can be completely eluted from the column by the buffer.

The complex is readily eluted from asbestos or cellulose columns by a dilute solution of a surface-active agent of the anionic or non-ionic type. The wavelength of maximum absorption by the eluted complex varies with different surface-active agents.

The volumes of reagents specified in the procedure for up to 30 μg of tin can be greatly reduced when working with about 1 μg. This speeds operations at the column separation stage, although coloured complex formation does not reach a maximum until about 3 hours after adding the reagents. (About 85 per cent. of the maximum is formed in 15 minutes.)

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In the procedure recommended for up to 30 μg of tin, final pH adjustment is made with ammonia solution. When using reduced volumes of reagents, this adjustment is undesirable because even small drops of 5 N ammonia solution give rise to large pH changes in a few millilitres of solution, and more dilute ammonia solutions cause excessive dilution, which leads to delayed coloured complex formation. Final pH adjustment is unnecessary if all tin solutions initially contain the same amount of acid and are therefore all brought to the required pH by the same volume of a suitable alkaline buffer. Tin solutions of equal acidity can be obtained by evaporating each tin solution in the presence of excess of sulphuric acid, and removing the excess of acid at 300° C. Each residue can then be re-dissolved in a known amount of acid. It was also found that with solutions prepared in this way the addition of alkali (as in the procedure for 30 μg of tin) before adding hydrochloric acid can be omitted.

The acid added and the buffer used as reagents for coloured complex formation affect the intensity of colour produced. Solutions of tin containing hydrochloric acid give higher intensities than solutions containing only sulphuric acid when used with an acetate-buffered system. When a phthalate-buffered system is used at Ross and White's[9] recommended pH value of 2·5, lower intensities are produced than those from the acetate system at the recommended pH of 3·8.

Calibration graphs for 0·2 to 1·6 μg of tin do not pass through the origin, nor are they completely linear, but they can be regarded as linear over small ranges (from 1 μg upwards to at least 1·6 μg the graphs are linear).

Reagents and materials—

All reagents should be of analytical-reagent grade.

Water, de-ionised.

Sulphuric acid, sp.gr. 1·84.

Sulphuric acid, N.

Hydrochloric acid, sp.gr. 1·18.

Dilute hydrochloric acid (1 + 2·2)—Dilute hydrochloric acid (sp.gr. 1·18) appropriately with water.

Catechol violet, 0·05 per cent. w/v, aqueous—Prepare fresh weekly.

Sodium acetate trihydrate - sodium hydroxide solution—Prepare a solution containing 40 g of sodium acetate trihydrate and 8·3 g of sodium hydroxide in 250 ml. Check this solution by adding 1 ml to a mixture of 0·3 ml of N sulphuric acid, 0·9 ml of water, 0·4 ml of dilute hydrochloric acid and 0·4 ml of catechol violet solution. If the resultant solution does not have a pH of 3·8, adjust the sodium acetate - sodium hydroxide solution by adding a solution of sodium acetate of equal concentration but with higher or lower sodium hydroxide concentration as necessary. (The difference in pH between two mixed solutions, one prepared with a sodium acetate - sodium hydroxide solution containing 8 g of sodium hydroxide per 250 ml of solution and the other with an acetate - hydroxide solution containing 0 g of sodium hydroxide per 250 ml, is about 0·2 pH unit.)

Sodium acetate trihydrate - hydrochloric acid solution—Prepare a 20 per cent. w/v aqueous solution of sodium acetate trihydrate and add sufficient hydrochloric acid to produce a pH of 3·8.

Asbestos fibres—Treat Gooch asbestos as follows. Slurry the asbestos with a dilute aqueous solution of the selected surface-active agent, by using a high speed mixer. Add distilled water to produce a dilute suspension of fibres, and allow all but the "fines" to settle. Decant. Repeat the diluting and decanting steps twice. Collect the asbestos on a Buchner funnel, and wash it with distilled water until it is free from surface-active agent. Dry the asbestos at 105° C.

Fibrous cellulose powder—A suitable column-chromatographic grade, *i.e.*, powder that disperses readily in aqueous solutions, settles freely and gives columns with high flow-rates, besides possessing adequate adsorptive capacity for the tin - catechol violet complex.

Surface-active agent, non-ionic or anionic, 0·5 per cent. v/v aqueous solution—Tergitol NPX (from B.D.H. Chemicals Ltd.) has been found satisfactory.

Tin(IV) stock solution—Dissolve 0·1000 g of pure tin in 20 ml of sulphuric acid, sp.gr. 1·84, by heating until fumes appear. Cool, cautiously dilute with 150 ml of water and cool again. Add 65 ml of sulphuric acid, sp.gr. 1·84, cool, and transfer to a 500-ml calibrated flask. Dilute to the mark with water (1 ml of solution = 200 μg of tin).

Tin(IV) dilute standard solution—Dilute 1·0 ml of tin(IV) stock solution with 30 ml of N sulphuric acid to 100 ml. Prepare just before use (1 ml of solution = 2 μg of tin).

PREPARATION OF CALIBRATION GRAPH—

Transfer to a series of 10-ml beakers, by pipette (or small-capacity burette), volumes of dilute standard tin solution to cover the whole or a suitable part of the range 0 to 1·6 μg of tin. Evaporate the solutions to dryness, first by heating on a steam-bath and finally by heating in a muffle furnace at 300° C until no sulphuric acid remains. Cool the beakers. Treat each as follows: add 0·3 ml of N sulphuric acid and warm on a steam-bath. With the aid of a glass rod, and by rotation of the beaker, ensure that the whole of the inside of the beaker is wetted with warm acid. Allow the solution to evaporate to the fullest extent that steam-bath heating conditions permit. Cool. Add, in order, without delay between each addition but with adequate mixing after each addition, 0·9 ml of water, 0·4 ml of dilute hydrochloric acid, 0·4 ml of catechol violet solution and 1·0 ml of sodium acetate - sodium hydroxide solution. Set aside for 3 hours.

Prepare asbestos or cellulose powder columns, one for use with each beaker, as follows: lightly plug with cotton-wool, to a distance of about 5 mm, the end of a piece of glass tubing of about 4 mm i.d. and not less than 150 mm long. Trim the plug so that no cotton-wool fibres protrude. Slurry 0·10 g of asbestos or 0·15 g of cellulose powder with a few millilitres of sodium acetate - hydrochloric acid solution and transfer all of the slurry to the plugged tube, by using additional solution as required. Allow the powder to settle and pack as freely as possible, disturbing it only if it becomes necessary to release any trapped air bubbles. When the excess of solution has drained through, the column is ready for use. The internal volume of the tubing above the column should be not less than 1·5 ml.

With a Pasteur pipette, transfer to the top of the column the whole of the solution that has been allowed to stand for 3 hours. Wash the 10-ml beaker with about 1·5 ml of sodium acetate - hydrochloric acid solution, and transfer the whole on to the column as soon as there is no more coloured solution above it. Allow the column to drain, and remove, by touching any hanging drop. Reject all column effluents. Without further delay add 1·5 ml of surface-active agent solution to the column to elute the tin - catechol violet complex and collect all of the 1·5 ml of eluate. With surface-active agent solution in a reference cell measure the optical density of the eluate in a 20-mm path micro cell, at the wavelength appropriate for the complex in the surface-active agent chosen (with Tergitol NPX, about 570 nm). Construct a graph for amount of tin *versus* optical density.

DETERMINATION OF ORGANOTIN STABILISER—

Of the two permitted organotin stabilisers for PVC food containers, only the mercaptoacetate compound referred to is extensively used in the U.K. The determination of this compound in vinegar, orange drink and cooking oil is dealt with below.

VINEGAR AND ORANGE DRINK

The tin stabiliser can be extracted from these foodstuffs by various chlorinated solvents and hydrocarbons, but well defined separation of solvent and foodstuff, after shaking them together to effect extraction, is not readily achieved even by centrifuging the mixture. Continuous extraction in an extractor of the type shown in Fig. 1 avoids this difficulty. Extraction with petroleum spirit is slower, but gives a cleaner extract than extraction with chlorinated solvents (for which a differently designed extractor was used).

Dilute solutions of stabiliser readily deposit organotin compounds on the surfaces of their containers. Consequently, when for the purpose of checking the recovery obtainable by a proposed analytical procedure a small amount of a dilute solution of stabiliser is added to a foodstuff, the weight of stabiliser then present in that solution must be determined. It cannot be calculated from the weight of stabiliser used for making the solution.

Organotin compounds deposited on the surface of the extraction flask during continuous extraction cannot readily be removed with simple common solvents, but their removal can be effected with a dilute solution of formic acid in, say, diethyl ether. A 2 per cent. formic acid (100 per cent.) solution in ether does not rapidly decompose the stabiliser. With higher concentrations the decomposition risk is increased.

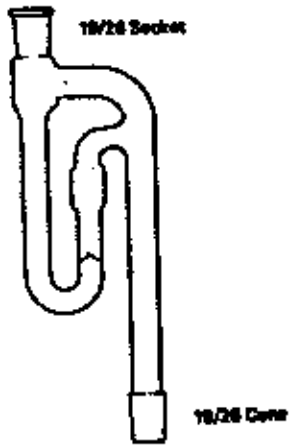

Fig. 1. Apparatus for continuous extraction (approximately one quarter of actual size)

Newman and Jones,[3] quoting Suk and Malat,[4] and Ross and White,[5] whose observations they confirm, list aluminium, gallium, indium, titanium, zirconium, thorium, antimony, bismuth, molybdenum, tungsten and iron as interfering species at the pH used for determining tin. Of these species, only aluminium and iron are likely to be present in foodstuffs, and these are separated from the mercaptoacetate organotin stabiliser by paper chromatography, with chloroform as developing solvent. Inorganic tin compounds are also separated from the stabiliser under the same conditions. The stabiliser travels in or just behind the solvent front. By double chromatographic development and the use of chromatographic paper cut to a special shape (Fig. 2) the stabiliser present in a few millilitres of petroleum spirit extract can be isolated within a paper area of 1 to 2 cm².

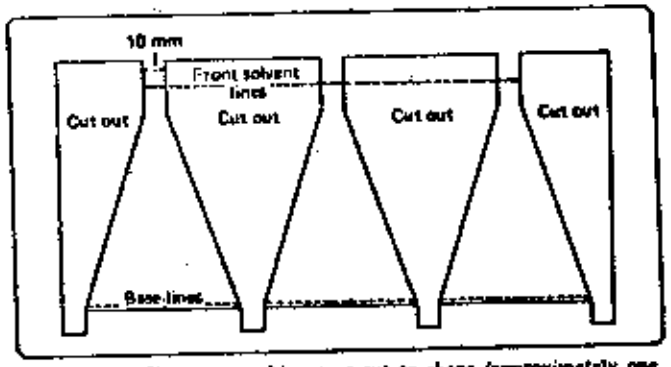

FIG. 2. Chromatographic paper cut to shape (approximately one third of actual size)

The area occupied by stabiliser can be established by spraying with 4-(2-pyridylazo)-resorcinol disodium salt (PAR), which produces a pink area on a yellow background.

The paper *plus* stabiliser can be oxidised with sulphuric acid - hydrogen peroxide mixture and the resulting solution evaporated to dryness at 300° C, to give a tin sulphate residue for which the previously described method for tin determination is appropriate.

REAGENTS AND APPARATUS—

Petroleum spirit, boiling range 60° to 80° C—Glass-distilled to minimise metallic contamination.

Formic acid, 98 to 100 per cent.—For preparation of a 2 per cent. v/v solution in ether.

Diethyl ether.

Chloroform.

4-(2-Pyridylazo)resorcinol, disodium salt (PAR), 0·5 per cent. w/v in ethanol.

Sulphuric acid, approximately 72 per cent. w/w—Dilute 64 ml of concentrated acid, sp.gr. 1·98, to 100 ml.

Hydrogen peroxide—Aristar grade.

Micro Kjeldahl flasks—Working capacity 0·5 to 0·7 ml. See procedure for orange drink extracts.

Continuous extractor—For extracting solvents lighter than the sample to be extracted. Sample capacity 5 ml.

Chromatographic paper—Whatman No. 1 or equivalent.

Pasteur pipette—The body consists of normal diameter glass tubing, joined to capillary tubing drawn out to produce the tip of the pipette.

Hair dryer or equivalent—This is used as a source of clean cold air.

PROCEDURE—

Extract 5 ml of vinegar (or orange drink) continuously with petroleum spirit for 2 hours, maintaining as fast a rate of extraction as possible. Concentrate the extract to about 1 ml, preferably at about 30° C, but do not allow it to evaporate to dryness.

Cut chromatographic paper to the shape shown in Fig. 2. With a capillary-ended Pasteur pipette, apply the concentrated extract to the base-line. Simultaneously, blow clean cold air on to the base-line area (preferably on to the underside of the paper) to minimise spread of the extract and to speed its evaporation. Continue blowing air until the paper is dry. Wash the extraction flask with two 0·2-ml portions of formic acid - ether solution and transfer the washings in a similar way to the base-line. Air dry the paper until it is substantially free from the smell of formic acid.

Develop the chromatogram with chloroform to the pre-drawn solvent front line. Dry the chromatogram in air and carry out a second development. After again removing the chloroform by exposure to air, spray the chromatogram with PAR solution. The pink area at the solvent front contains the stabiliser. Cut out this area.

Vinegar extracts—For these extracts place the cut-out paper in a 10-ml beaker. Add 0·5 ml of 75 per cent. w/w sulphuric acid and warm on a steam-bath to disperse, and lightly char the paper. Add two drops of Aristar hydrogen peroxide and allow them to react. Add further peroxide, a few drops at a time as necessary, to produce a colourless solution. Evaporate the solution first to as small a volume as possible at 105° C, then to dryness at 300° C. (If the solution becomes coloured during evaporation, cool the beaker slightly, add 1 drop of peroxide and, after a few minutes, continue the evaporation.) For the determination of the tin in the residue in the beaker, proceed as given under Preparation of calibration graph from "Cool the beakers. Treat each as follows . . ."

Orange drink extracts—In these extracts orange oil will be present in the cut-out paper. This reacts too vigorously with sulphuric acid - hydrogen peroxide for this treatment to be conducted in an open beaker without risk of loss by splashing. Hence, carry out the oxidation in a micro Kjeldahl flask (made by blowing a bulb at the end of a 12 × 100-mm Pyrex test-tube). Continue treatment until a colourless solution is maintained while sulphuric acid fumes are being driven off. Transfer the cooled solution to a 10-ml beaker with a Pasteur pipette. Wash the flask and pipette with two 0·2-ml portions of x sulphuric acid and add the washings to the 10-ml beaker. Evaporate the solution at 105° C, then heat to dryness at 300° C, and proceed to the determination of tin as given under Preparation of calibration graph from "Cool the beakers. Treat each as follows . . ."

COOKING OIL (SUNFLOWERSEED OIL)

Silica gel is a convenient adsorbent for the stabiliser. We were advised (Mr. S. Greenfield of Albright & Wilson Ltd., in a private communication) that silica-gel columns are suitable for the adsorption of dialkyltin dilaurates from solutions of these compounds in Light Liquid Paraffin B.P. It was found that the mercaptoacetate compound can be similarly adsorbed from solution in sunflowerseed oil. The adsorbed tin stabiliser can be eluted from the silica-gel columns by formic acid - ether solution.

Silica gel also adsorbs certain other tin compounds commonly present in the mercaptoacetate, which are not removed by formic acid - ether solution. Thus when assessing analytical procedures that include a silica gel adsorption step for the determination of the mercaptoacetate stabiliser, the weight of stabiliser taken for dissolution in oil cannot be used as a basis for the calculation of percentage recovery of mercaptoacetate. The percentage purity of the stabiliser, as determined by passing a solution of it in, say, petroleum spirit through a silica-gel column, must be included in the calculation.

REAGENTS AND APPARATUS—

As for vinegar and orange drink, but omitting the continuous extractor and adding the following reagent.

Silica gel—Chromatographic grade, particle size 0·05 to 0·20 mm (*e.g.*, Merck No. 7734), activated at 150° C for 2 hours.

PROCEDURE—

Dilute 5 ml of cooking oil by addition of 15 ml of petroleum spirit.

Set up a column of silica gel as follows. Lightly plug the end of a piece of glass tubing, 4 mm i.d. × 150 mm long, with cotton-wool. Seal the plugged end of the tube with a finger tip and almost fill the tube with petroleum spirit, then pour in 0·15 g of silica gel. Remove the finger tip from the end of the tube to allow column formation.

Pass the diluted oil sample through the column. Wash the column with 1 ml of petroleum spirit and three 1-ml portions of ether, allowing each 1-ml portion to disappear into the column before adding the next portion. Reject all of the column effluents and wipe residual traces of oil from the lower end of the glass tube.

Elute the stabiliser into a small flask by passage of four 1-ml portions of formic acid - ether solution through the column. Transfer the eluate to the base-line of specially cut chromatographic paper. Wash the flask with two 0·2-ml portions of formic acid - ether solution and transfer the washings to the paper also. Continue as in the orange drink extracts procedure from "Air dry the paper until it is substantially free from the smell of formic acid . . ."

RESULTS OBTAINED FROM PREPARED SOLUTIONS OF STABILISER IN THE THREE FOODSTUFFS

VINEGAR—

Solutions of the stabiliser in glacial acetic acid were prepared, and used without delay, for addition to malt vinegar in proportions sufficiently small to cause no significant change in the acetic acid content of the vinegar. For example, 0·08 to 0·24 g of stabiliser (approximate tin content 15 per cent.) was dissolved in 50 ml of glacial acetic acid and 2 μl of the solution added to 5 ml of vinegar.

ORANGE DRINK—

Solutions of the stabiliser in orange oil were prepared and added in a commercially appropriate proportion to an orange drink from which the orange oil normally added had been omitted. The oil was thoroughly mixed with the drink by vigorous agitation in the continuous extractor, in readiness for the start of the tin determination. For example, 0·03 to 0·09 g of stabiliser was dissolved in 50 ml of orange oil and 5 μl of the solution added to 5 ml of oil-free orange drink.

COOKING OIL—

Solutions of stabiliser were prepared in cooking oil and added in small proportions to cooking oil. For example, 0·04 to 0·12 g of stabiliser was dissolved in 50 ml of oil and 4 μl of the solution added to 5 ml of oil.

A solution of stabiliser in petroleum spirit was also prepared, and tin determined in (*i*) the whole solution, (*ii*) the stabiliser isolated by the paper-chromatographic step and (*iii*) the portion of the stabiliser isolated by both the silica gel and paper-chromatographic steps.

For vinegar the average recovery, from the mean of three replicates, at various tin concentrations from 0·4 to 1·5 μg per 5 ml, was 75 per cent. and precision ($\pm ts/\sqrt{n}$) 10 per cent. For orange drink the corresponding figures were 90 and 6 per cent., and for cooking oil 75 and 12 per cent. Recoveries were not statistically different at different tin concentrations. For all three foodstuffs zero recoveries were obtained when no organotin was added.

The stabiliser decomposes under aqueous acidic conditions. Failure to obtain 100 per cent. recovery of stabiliser added to foodstuff may therefore be caused by its partial decomposition, either on addition to the foodstuff or during the isolation procedures.

CALCULATION OF TIN STABILISER CONTENT OF FOODSTUFF—

The stabiliser content, in parts per million of foodstuff that has been stored in a PVC container stabilised with the mercaptoacetate compound approved by the American Food and Drugs Administration, can be calculated from the figure for tin derived from organotin by use of the percentage recovery figures quoted, and application of factors for the permitted tin content of the stabiliser and the specific gravity of the foodstuff.

We thank the Director of the Research Association for the Paper and Board, Printing and Packaging Industries for permission to publish this paper.

REFERENCES

1. Analytical Methods Committee, *Analyst*, 1967, 92, 322.
2. Ross, W. J., and White, J. C., *Analyt. Chem.*, 1961, 33, 421.
3. Newman, E. J., and Jones, P. D., *Analyst*, 1966, 91, 406.
4. Suk, V., and Malat, M., *Chemist Analyst*, 1956, 45, 30.

Received *February 6th*, 1970
Accepted *May 1st*, 1970

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PART 121—FOOD ADDITIVES

Subpart D--Food Additives Permitted in Food for Human Consumption

STANNOUS CHLORIDE

The Commissioner of Food and Drugs, having evaluated the data in a petition (FAP 7J2090) filed by The P.J. Ritter Co., Bridgeton, N.J. 08302, and other relevant material, has concluded that a food additive regulation should issue to prescribe the safe use of stannous chloride for color retention in asparagus packed in glass with lids lined with an inert material. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c) (I)). 72 Stat. 1786; 21 U.S.C. 348(c)(1)) and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (21 CFR 2.120), Part 121 is amended by adding to Subpart D the following new section:

121.1213 Stannous chloride.

The food additive stannous chloride may be safely used for color retention in asparagus packed in glass, with lids lined with an inert material, in an amount not to exceed 20 parts per million calculated as tin (Sn).

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the Federal Register file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 6440, 330 Independence Avenue, SW., Washington, D.C. 20201, written objections thereto, preferably in quintuplicate. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularly the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state

the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought objections may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective on the date of its publication in the Federal Register.

(Sec. 400(c)(1)), 72 Stat. 1786, 21 U.S.C. 348(c)(1))

Dated December 26, 1967

R.E. Duggan

Acting Associate Commissioner for Compliance

(F.R. Doc. 68-100, Filed Jan 3, 1968; 8:48 am)

 Analyst, May, 1967, Vol. 92, *pp.* 320 :

Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Tin in Organic Matter

Part I: Amounts of Tin up to 30 μg

THE Analytical Methods Committee has received the following Report from its Metal Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. W. C. Johnson (Chairman), Dr. J. C. Gage, Dr. T. T. Gorsuch (resigned November, 1966), Dr. R. A. Hoodless, Miss E. M. Johnson, Dr. H. Liebmann, Dr. R. F. Milton, Dr. E. Newman and Mr. G. E. Thackray, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

Tin may be present in organic materials, such as foodstuffs, in concentrations ranging from less than one to several hundred parts per million. The Sub-Committee has considered several colorimetric, titrimetric and polarographic methods for the determination of small amounts of tin, and has concluded that no single method could conveniently be adopted for the determination of such a wide range of tin concentrations in organic matter. The Sub-Committee's investigation has therefore been divided into the examination of methods for determining tin in two separate ranges of concentration. For amounts of tin up to 30 μg a colorimetric method based on the reaction of tin(IV) with catechol violet is recommended; the other method under consideration is the colorimetric method involving the use of the zinc complex of toluene-3,4-dithiol for amounts in the range 30 to 150 μg. For amounts above 150 μg a method involving titration of tin(II) with iodine would undoubtedly prove suitable.

METHOD A: FOR AMOUNTS OF TIN NOT GREATER THAN 30 μg

The method recommended is that of Newman and Jones,[1] which is based on, but contains modifications of, the procedures of Tanaka[2] and Tanaka and Yamayoshi.[3] This method was chosen because it contains a solvent-extraction stage designed for the selective extraction of tin from sulphuric acid solutions such as would be produced from wet oxidations of organic matter.

EXPERIMENTAL—

In view of the work that had been carried out on behalf of the Analytical Methods Committee by Newman and Jones,[1] the Sub-Committee decided that the usefulness or otherwise of the method could be demonstrated adequately by having its members carry out a simple collaborative test.

A sample of orange squash was divided between the collaborating laboratories. Each laboratory determined the tin content of the sample by the recommended method after wet oxidation with nitric and sulphuric acids, or with 50 per cent. hydrogen peroxide and sulphuric acid. Recovery tests were also conducted in which amounts of tin equivalent to 140, 240 and 1040 p.p.m. were added to the sample before wet oxidation. The tin was added

quotions of the dilute standard tin solution (tin(IV) sulphate) prepared as described under "Method" in the Appendix to this Report. In one laboratory, tin was also added as tin(IV) chloride to find out whether volatilisation losses of tin(IV) chloride could occur during the early stages of the digestion.

The results obtained are shown in Table I. The figures for the tin content of the sample are in good agreement and the recoveries of added tin were all satisfactory.

It was found by two of the collaborating laboratories during preliminary experiments with the orange squash that reproducible results were obtained only when completely colourless solutions were produced.

TABLE I

DETERMINATION OF TIN IN ORANGE SQUASH

Laboratory	Tin added, p.p.m.	Tin found, p.p.m.	Tin recovered, p.p.m.
A	0	2·0	—
	1·0	3·0	1·0
	5·0	6·7	4·7
	10·0	10·9	8·9
	0	2·2	—
	1·0	3·2	1·0
	5·0	6·8	4·6
	10·0	11·9	9·7
B	0	2·1	—
	0	2·1	—
	1·0	3·3	1·1
	1·0	3·1	1·2
	5·0	7·8	5·6
	5·0	7·8	5·6
	10·0	12·3	10·1
	10·0	12·2	10·0
C	0	2·3	—
	0	2·2	—
	0	2·1	—
	1·0*	3·3	1·1
	1·0*	3·1	0·9
	1·0*	3·3	1·1
	5·0*	7·0	4·8
	5·0*	7·2	5·0
	5·0*	7·3	5·1
	10·0*	12·5	10·3
	10·0*	11·8	9·6
	10·0*	12·1	9·9
D‡	0	2·7	—
	0	2·7	—
	1·0	3·6	0·9
	1·0	3·6	0·9
	5·0	8·1	5·4
	5·0	7·7	5·0
	10·0	13·6	10·9
	10·0	13·1	10·4
	0	2·5	—
	0	2·3	—
	1·0	3·9	0·9
	1·0	3·5	1·1
	5·0	7·0	4·0
	5·0	7·3	4·9
	10·0	11·8	9·4
	10·0	12·6	10·2

* Tin added as the chloride.

† Tin added as the sulphate.

‡ The two sets of results quoted were obtained with different supplies of catechol violet. A separate calibration graph was prepared for each.

Appendix

Recommended method for the determination in organic matter of amounts of tin not greater than 30 μg

Principle of method—

After destruction of the organic matter by wet oxidation with nitric and sulphuric acids,[1] nitric, perchloric and sulphuric acids[1] or 50 per cent. hydrogen peroxide in the presence of sulphuric acid,[2] the residual sulphuric acid is diluted to four times its volume with water to give an approximately 9 N concentration of the acid.

Tin is selectively separated from this solution by treating it with potassium iodide and extracting tin(IV) iodide into toluene. Tin(IV) is then returned to aqueous solution by shaking the toluene extract with a solution of sodium hydroxide. After acidification, and removal of free iodine from the solution, the tin(IV) is determined spectrophotometrically as its coloured complex with catechol violet, the solution being buffered to pH 3·8 with acetate.

Range—

For tin contents in the range 1 to 30 μg in the sample taken.

Applicability—

The colour reaction between tin(IV) and catechol violet is far from selective. However Newman and Jones[3] have shown that the solvent-extraction step is highly selective or even specific. For the present application, therefore, the recommended method can be regarded as specific.

Reagents—

All reagents should be of analytical grade.

Water. Purify glass-distilled water further by passing it through a mixture of strongly acidic cation exchange resin and strongly basic anion-exchange resin.

Sulphuric acid, approximately 9 N. Cautiously mix 250 ml of sulphuric acid, sp.gr. 1·84 with 500 ml of water, cool to room temperature, and dilute to 1 litre with water.

Potassium iodide, approximately 5 M.—Dissolve 83 g of potassium iodide in water to produce 100 ml. Prepare freshly each day.

Toluene (free in benzene).

Sodium hydroxide, approximately 5 N and approximately 0·1 N.

Hydrochloric acid, approximately 5 N.

Ascorbic acid solution.—A freshly prepared 5 per cent. w/v aqueous solution.

Catechol violet solution.—A 0·05 per cent. w/v aqueous solution. Prepare freshly each week.

Sodium acetate trihydrate solution.—A 20 per cent. w/v aqueous solution.

Ammonia solution, approximately 5 N.

Tin(IV) stock solution.—Dissolve 0·1000 g of pure granulated tin in 20 ml of sulphuric acid, sp.gr. 1·84, by heating until fumes appear. Cool, cautiously dilute with 150 ml of water, and cool again. Add 65 ml of sulphuric acid, sp.gr. 1·84, cool, and transfer to a 500-ml calibrated flask. Dilute to the mark with water.

1 ml of solution = 200 μg of tin.

Tin(IV) dilute standard solution.—Dilute 50 ml of tin(IV) stock solution to 100 ml with water in a calibrated flask. Prepare freshly each day.

1 ml of solution = 10 μg of tin.

Preparation of calibration graph—

Transfer by pipette, or small-capacity burette, suitable volumes of dilute standard tin solution, to cover the range 0 to 30 μg of tin, to a series of 50 ml beakers and treat each as follows: dilute to 7 ml with water, add 1 ml of 5 N sodium hydroxide, and mix. Add 2·5 ml of 5 N hydrochloric acid, mix, add 2·0 ml of catechol violet solution, mix again, and add 5 ml of sodium acetate solution (see Note 1). Adjust the pH of the solution with 5 N ammonia solution to 3·8 ± 0·1 units, with the aid of a pH meter. Transfer to a 25-ml calibrated flask, dilute to the mark with water, mix thoroughly, and set aside for 30 minutes. Measure

optical density of the solution in a 1-cm cell at a wavelength of 552 mμ, with the solution containing no added tin in the reference cell. Construct a graph relating the amount of tin to the optical density (see Note 2). The graph should be rectilinear and pass through the origin.

PROCEDURE—

Dilute the sulphuric acid solution containing not more than 30 μg of tin to approximately 15 ml, cool, and transfer it to a separating funnel. For each 25 ml of solution add 2·5 ml of 5 M potassium iodide, mix, and add 10 ml of toluene. Insert the stopper, shake the funnel vigorously for 2 minutes, allow the layers to separate, and discard the aqueous phase. Wash the toluene layer, without shaking it, with 5 ml of a solution prepared by mixing 25 ml of 18 N sulphuric acid and 2·5 ml of 5 M potassium iodide, and discard the washings. The toluene layer will be coloured pink with extracted iodine.

Add 5 ml of water to the toluene extract and then 5 N sodium hydroxide dropwise, with shaking, until the toluene layer is colourless. Add 2 drops of 5 N sodium hydroxide in excess (usually a total of 8 to 10 drops is required). Insert the stopper, shake the funnel for 30 seconds, allow the phases to separate, and transfer the aqueous layer into a 50-ml beaker. Shake the toluene layer with 3 ml of 0·1 N sodium hydroxide for 30 seconds, allow the layers to separate, and add the aqueous layer to the contents of the 50-ml beaker. Retain the organic (toluene) layer.

Acidify the aqueous solution in the beaker with 2·5 ml of 5 N hydrochloric acid, and **hydrolyse** the liberated iodate by the dropwise addition of ascorbic acid solution. Add 2·0 ml pyrocatechol violet solution, and mix. Wash the toluene retained from above, without shaking, with 5 ml of sodium acetate solution. Add the washings to the contents of the beaker, mix, and adjust the pH of the solution to 3·8 ± 0·1 units with 5 N ammonia solution by means of a pH meter. Transfer the solution to a 25-ml calibrated flask, and complete the determination of tin as described above under "Preparation of Calibration Graph." Calculate the amount of tin present by reference to the calibration graph.

NOTES—

1. The order of addition of reagents is important, and the stated order should be strictly adhered to.

2. When a new bottle or batch of catechol violet is used a fresh calibration graph should be prepared.

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1. Newman, E. J., and Jones, P. H., *Analyst*, 1966, **91**, 106.
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4. Analytical Methods Committee, *Analyst*, 1960, **85**, 643.
5. ——, "The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter," *Ibid.*, 1967, **92**, in the press.

Analyst, June, 1968, Vol. 93, pp. 411 to

Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Tin in Organic Matter

Part II*: Amounts of Tin from 30 to 150 μg

THE Analytical Methods Committee has received the following Report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. W. C. Johnson (Chairman), Dr. J. C. Gage, Dr. T. T. Gorsuch (resigned November, 1966), Dr. R. A. Hoodless, Miss E. M. Johnson, Mr. D. A. Lambie (appointed January, 1967), Dr. H. Liebmann, Dr. R. F. Milton, Dr. E. J. Newman and Mr. G. B. Thackray, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

The Sub-Committee has previously recommended a method for determining up to 30 μg of tin in organic matter.[1] As tin can be present in organic matter, particularly foodstuffs, over a fairly wide range of concentrations, a method for determining amounts larger than 30 μg was required. In consequence, the Sub-Committee has investigated a colorimetric method involving the use of the zinc complex of toluene-3,4-dithiol. This method has been found to be satisfactory, and is recommended for the determination of tin from 15 p.p.m. upwards. Full details of the method are given in the Appendix to this Report.

METHOD B: FOR AMOUNTS OF TIN FROM 30 TO 150 μg

The method recommended is based on the modification of Clark's method[2,3] proposed by Ovenston and Kenyon.[3,4] Sodium lauryl sulphate is used as dispersing agent preventing coagulation of the red tin - dithiol complex.

EXPERIMENTAL—

The Sub-Committee decided to test the method by carrying out a collaborative exercise with a sample of dried carrots as the organic matter. Each laboratory carried out a series of recovery experiments by adding known amounts of tin to the sample before wet oxidation. The tin was added as aliquots of a standard tin solution. The results obtained are shown in Table I. Laboratory B also carried out some recovery experiments in which flour was used as the organic matter. The results obtained are shown in Table II.

It was found that erratic results were obtained if the acid concentration in the colorimetric solution varied appreciably from the equivalent of 1 ml of concentrated sulphuric acid in 20 ml of solution.

Variable results were also obtained unless the amount of nitric acid used for the digestion was kept to a minimum, and all the nitric acid was removed from the final sulphuric acid residue.

* For details of Part I of this series, see reference list, p. 416.

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TABLE I

RECOVERY OF TIN FROM 10-g PORTIONS OF DRIED CARROTS

Laboratory	Tin added, p.p.m.	Tin recovered, p.p.m.
A	15·0[a]	15·2
	15·0[c]	13·6
	15·0[a]	16·2
	30·0[c]	30·4
	30·0[a]	30·4
	30·0[c]	31·0
B	15·0[b]	19·0
	15·0[a]	18·5
	15·0[a]	16·3
	30·0[a]	33·5
	30·0[a]	33·0
	30·0[b]	31·3
C	10·0[b]	9·5
	15·0[c]	14·5
	15·0[c]	14·5
	30·0[c]	28·0
	30·0[c]	30·0
	30·0[a]	29·0

Samples marked "a" were wet oxidised with nitric, perchloric and sulphuric acids.
Samples marked "b" were wet oxidised with nitric and sulphuric acids.
Samples marked "c" were wet oxidised with 50 per cent. w/v hydrogen peroxide and sulphuric acid.

TABLE II

RECOVERY OF TIN FROM 10-g PORTIONS OF FRUIT PULP

Tin added, p.p.m.	Tin recovered, p.p.m.
15·0	13·7
15·0	13·5
30·0	28·7
30·0	29·5

Appendix

RECOMMENDED METHOD FOR THE DETERMINATION IN ORGANIC MATTER OF AMOUNTS OF TIN FROM 30 TO 150 μg

DISCIPLE OF METHOD—

The organic matter in the sample is destroyed by wet oxidation with nitric and sulphuric ids, with nitric, perchloric and sulphuric acids,[6] or with 50 per cent. w/v hydrogen peroxide a free) and sulphuric acid.[7] The residue is diluted with water and extracted with a solution dithizone in carbon tetrachloride to remove any copper present. The aqueous solution then allowed to react with zinc dithiol in the presence of thioglycollic acid, and sodium ryl sulphate, which acts as a dispersing agent for the tin - dithiol complex. The extinction the red-coloured suspension is measured at 535 mμ.

INGE—

The aliquot of the sample solution taken should contain between 30 and 150 μg of tin.

AGENTS—

All reagents should be of analytical-reagent quality unless stated otherwise.

Dithizone solution—Prepare a 0·02 per cent. w/v solution in carbon tetrachloride. This ation should be recently prepared or stored in a refrigerator.

Sodium lauryl sulphate solution—Prepare a 1 per cent. w/v aqueous solution using dium lauryl sulphate of B.P. quality.

Sulphuric acid, 20 per cent. v/v—To 50 ml of water cautiously add 20 ml of concentrated phuric acid (sp.gr. 1·84). Cool and dilute to 100 ml.

Tin stock solution—Dissolve 0·100 g of pure granulated tin in 20 ml of sulphuric acid gr. 1·84) by heating until fumes appear. Cool, cautiously dilute with 150 ml of water, l cool again. Add 65 ml of sulphuric acid (sp.gr. 1·84), again cool, and transfer to a 500-ml brated flask. Dilute to the mark with water.

Tin standard solution—Dilute 10 ml of tin stock solution to 100 ml with water. Prepare freshly each day. (1 ml of solution = 20 μg of tin).

Zinc dithiol—Dissolve 0·2 g of zinc dithiol in 1 per cent. sodium hydroxide solution containing a few drops of ethanol. Add 1 ml of thioglycollic acid and dilute to 100 ml with 1 per cent. sodium hydroxide solution. Prepare immediately before use.

PROCEDURE—

Destroy the organic matter in an appropriate amount of the sample by wet oxidation with sulphuric and nitric acids; sulphuric, perchloric and nitric acids; or sulphuric acid and 50 per cent. w/v hydrogen peroxide. When oxidation is complete, dilute the solution with 10 ml of water and boil gently to fuming. Allow the solution to cool, add a further 10 ml of water and boil gently to fuming. Transfer the final clear solution to a calibrated flask of such volume that the diluted solution contains no more than the equivalent of 4 ml of concentrated sulphuric acid per 100 ml.

Transfer, by pipette, 10 ml of this solution containing between 30 and 150 μg of tin into a separating funnel, add 5 ml of dithizone solution, and shake the funnel. Allow the layers to separate, and discard the lower dithizone layer. Continue the extraction with successive 5-ml portions of dithizone solution until the extracts remain green. Wash the aqueous solution with two successive 5-ml portions of carbon tetrachloride, and discard the washings.

Transfer the aqueous phase to a 20 ml calibrated flask and add 20 per cent. sulphuric acid, so that the final solution contains the equivalent of between 0·7 and 1 ml of concentrated sulphuric acid. Add 1 ml of sodium lauryl sulphate solution, mix, and then add 1 ml of zinc dithiol reagent.

Dilute to the mark with water, mix thoroughly, and immerse in a boiling water bath for exactly 1 minute. Allow the solution to cool at room temperature for 20 to 30 minutes and then measure the extinction of the solution at a wavelength of 535 mμ, by using 1-cm cells with a reagent blank solution in the comparison cell.

Read the number of micrograms of tin equivalent to the observed extinction from a previously prepared calibration graph, and calculate the tin content of the sample.

PREPARATION OF CALIBRATION GRAPH—

Transfer aliquots of standard tin solution to cover the range 30 to 150 μg of tin to a series of 20-ml calibrated flasks. Add 5 ml of 20 per cent. sulphuric acid, mix, and proceed as described above beginning at "Add 1 ml of sodium lauryl sulphate solution. . . ."

Measure the extinctions of the solutions, and construct a graph relating the extinctions to the number of micrograms of tin.

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NOTE—Reference 1 is to Part I of this series.

THE EFFECT OF TIN MICROELEMENT ON CHOLESTEROL AND LECITHIN LEVEL IN RABBIT BLOOD SERUM

by

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We know that tin comprises food products of both animal and vegetable origin, and is also a regular composite part of human and animal organisms [1-4].

In spite of the appreciable propagation of tin in living matter, its biological function in the organism has not been fully studied [5].

The aim of our study was to explain how various doses of tin affect the cholesterol and lecithin level in rabbit blood serum.

Material and Method

Tests were run on female rabbits weighing 2-3kg. The animals were divided into two groups and were kept on a regular food diet. Animals of both groups were injected daily with stannous chloride per os for a period of 15 days: rabbits of one group (allowing for pure metal) - 0.6mg per kilogram body weight; rabbits of the other group - 3mg. Blood serum was taken for

analysis prior to commencement of the test and thrice during the test period: on the 5th, 10th, and 15th days.

The level of cholesterol was determined by using a photoelectrocolorimeter via the Liebermann-Burchhardt color reaction.

In order to determine lecithin content, blood serum phosphor-lipids were extracted by the Ostrovsky method [6]. The lecithin level was determined by the amount of phosphorus [7]. The results were subjected to statistical analysis [8].

Test Findings and Considerations

We can see from Table 1, which shows data pertaining to the effect of tin on the cholesterol level after various time intervals, that on the fifth day after injection of the microelement, the amount of cholesterol in the blood serum diminishes in comparison with control data. This was especially noticeable on the tenth day. Similarly, by varying the cholesterol content and injecting a large dose of tin - 3mg.

At the end of the test, i.e., on the 15th day, the cholesterol level was almost back to normal. Cited

Table 1

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

Time after injection of tin	0.6mg				3mg			
	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

data attest that injections of both 0.6 and 3mg of tin lead to a reduction of the cholesterol in rabbit blood serum, mainly during the first ten days of testing.

Table 2

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

Time after injection of tin	0.6mg				3mg			
	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

Test findings pertain to the effect of tin on lecithin level in blood serum (Table 2). On the 5th and 10th days the amount of lecithin drops, and on the 15th day is almost back to normal. Lecithin content drops less significantly in comparison to cholesterol.

Our resultant data lead to the notion that the biological function of tin in the organism can surely be associated with the exchange of lipids.

Conclusions

1. Injections of tin in doses of 0.6 and 3mg on 5th and 10th days led to reduction in cholesterol and lecithin level in blood serum of rabbits.

2. On 15th day of test after injection of 3mg of tin, lecithin and cholesterol levels return to normal.

3. Resultant data attest that microelement tin participates in exchange of lipids in the animal organism.

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УДК 577.1:612.015.32:547.915

ВПЛИВ МІКРОЕЛЕМЕНТУ ОЛОВА НА ВМІСТ ХОЛЕСТЕРИНУ І ЛЕЦИТИНУ В СИРОВАТЦІ КРОВІ КРОЛІВ

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(Надійшла до редакції 17.VI 1967 р.)

Відомо, що олово входить до складу харчових продуктів як тваринного, так і рослинного походження, а також є звичайною складовою частиною організму людини і тварин [1—4].

Незважаючи на значне поширення олова в живій природі, біологічна роль його в організмі зовсім не вивчена [5].

Метою нашого дослідження було з'ясувати, як впливають різні дози олова на вміст холестерину й лецитину в сироватці крові кролів.

Матеріал та методика

Досліди проводили на кролях-самцях вагою 2—3 *кг*. Всіх тварин було поділено на дві групи, утримувались вони на звичайному харчовому раціоні. Тваринам обох груп щоденно вводили хлористе олово *per os* протягом 15 днів, кролям першої групи (з розрахунку на чистий метал) — 0,6 *мг* на 1 *кг* ваги тіла, другої — 3 *мг*. Сироватку крові для аналізу брали перед початком досліду і тричі протягом дослідного періоду — на 5, 10 та 15-й день.

Вміст холестерину визначали за допомогою фотоелектроколориметра з використанням кольорової реакції Любермана—Бурхарда.

Для визначення лецитину фосфоліпіди сироватки крові екстрагували за методом Островського [6]. Вміст лецитину визначали за кількістю фосфору [7]. Одержані дані піддавали статистичній обробці [8].

Результати дослідів та їх обговорення

З табл. 1, в якій наведено дані щодо впливу олова на вміст холестерину через різні проміжки часу, видно, що на п'ятий день після введення цього мікроелементу кількість холестерину в сироватці крові порівняно з даними контролю знижується. Особливо це помітно на 10-й день.

Так само змінювався вміст холестерину і при введенні більшої дози олова — 3 *мг*.

Таблиця 1

Вміст холестерину в сироватці кролів при введенні різних доз олова (в *мг*%)

Час після введення олова	0,6 *мг*				3 *мг*			
	n	*M*	*p*	%	*n*	*M*	*p*	%
Норма (контроль)	9	64,4		100	10	57,6		100
5-й день	9	40	<0,01	62,1	10	30,1	>0,001	52,1
10-й »	9	31,7	<0,01	49,2	10	27,8	>0,001	48,2
15-й »	9	42,2	<0,02	65,5	10	51,8	>0,5	89,9

Наприкінці досліду, тобто на 15-й день, вміст холестерину відновлювався майже до норми. Наведені дані свідчать про те, що введення як 0,6, так і 3 *мг* олова призводить до зменшення вмісту холестерину в сироватці крові кролів головним чином протягом перших десяти днів досліду.

Таблиця

Вміст лецитину в сироватці крові кролів при введенні різних доз олова (в *мг*%)

Час після введення олова	0,6 *мг*				3 *мг*			
	n	*M*	*p*	%	*n*	*M*	*p*	%
Норма (контроль)	10	175,5		100	10	151		100
5-й день	9	151,6	<0,1	86,3	9	117,2	<0,01	77,[illegible]
10-й »	10	140	<0,01	79,7	9	107,2	<0,01	70,[illegible]
15-й »	9	169,1	<0,5	96,5	7	150,7	>0,5	99,[illegible]

Результати дослідів щодо впливу олова на вміст лецитину в сироватці крові представлено в табл. 2. На п'ятий та десятий день кількість лецитину зменшується, а на 15-й день майже повертається до норми. Вміст лецитину порівняно до холестерину знижується менш істотно.

Одержані нами дані наводять на думку, що біологічна роль олова в організмі може бути певною мірою пов'язана з обміном ліпідів.

Висновки

1. Введення олова в дозі 0,6 і 3 *мг/кг* на 5 і 10-й день призводить до зниження вмісту холестерину та лецитину в сироватці крові кролів.

2. На 15-й день досліду після введення 3 *мг* олова вміст лецитину і холестерину повертається до норми.

3. Одержані дані свідчать про те, що мікроелемент олово бере участь в обміні ліпідів у тваринному організмі.

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ВЛИЯНИЕ МИКРОЭЛЕМЕНТА ОЛОВА НА СОДЕРЖАНИЕ ХОЛЕСТЕРИНА И ЛЕЦИТИНА В СЫВОРОТКЕ КРОВИ КРОЛИКОВ

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Резюме

Исследовали влияние разных доз микроэлемента олова на содержание холестерина и лецитина в сыворотке крови кроликов. Установлено, что олово, введенное в дозах как 0,6, так и 3 *мг*, приводит к снижению содержания холестерина и лецитина на 5 и 10-й дни опыта. На 15-й день под влиянием 3 *мг* олова уровень холестерина и лецитина восстанавливается до нормы.

EFFECT OF TIN MICROELEMENT ON CHOLESTEROL AND LECITHIN CONTENT IN RABBITS' BLOOD SERUM

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Summary

An experimental investigation was carried out to determine the effect of different doses of the tin microelement on the cholesterol and lecithin content in the rabbits blood serum.

It has been determined that the tin dose both of 0.6 mg and of 3 mg causes a decrease in the content of cholesterol and lecithin on the 5th—10th day.

On the 15th day under the effect of 3 mg of tin the cholesterol and lecithin level restored to norm.

this figure would provide a considerable safety margin for pears canned as halves which are normally heated for 20–30 min at about 100°, and it would inevitably exclude a considerable number of pears that would not discolour under commercial processing.

The experimental techniques outlined above, therefore, have more value in the study of the variation in the leucocyanidin content of pears and in the comparison of their behaviour on heating than in the selection of raw material for canning purposes.

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PINK DISCOLORATION IN CANNED PEARS
III.*—Inhibition by chemical additives

By B. V. CHANDLER and K. MARY CLEGG†

Despite the identification of the pigment responsible for discoloration in canned pears as a tin cyanidin complex, addition of stannous ions to susceptible pear purée prior to processing partly or completely inhibits the discoloration. This effect cannot be obtained with canned pear halves, but other reducing agents, such as sulphur dioxide, are effective inhibitors of the discoloration. By such treatment canned pears of acceptable quality can be produced from varieties which are normally avoided for processing because of their susceptibility to discoloration. A mechanism whereby reducing agents prevent the formation of pink pigments is described, and an explanation is given of the differences in the effect of stannous ions on pear halves and pear purées.

Introduction

It was previously reported[1] that the 'pink' pigment in discoloured canned pears is a purple-pink insoluble tri-anthocyanin complex derived from reaction between stannous ions from can corrosion and cyanidin-like compounds from heat-catalysed degradation of pear leucocyanidins. However, during attempts to prevent discoloration by creating conditions unfavourable to complex formation, there were indications that a preliminary treatment to promote can corrosion prior to leucocyanidin break-down might control discoloration in borderline cases, even though the introduction of stannous ions into the sample by tin corrosion should favour complex formation. This paper confirms the inhibition of discoloration by tin ions under certain circumstances, indicates a possible mechanism for the inhibition by such reducing agents, and presents a method for preparing canned pears of satisfactory colour and flavour from varieties which normally undergo considerable discoloration during processing.

Experimental and Results

In this work the use of pear purée and the distribution of pear quarters among four processing treatments eliminated as far as possible variations arising from the considerable differences in the leucocyanidin content of individual pears.[2] Peeled and cored pear quarters were processed in a 27·5% (by wt.) sucrose syrup with a flesh to syrup ratio of 180:110 parts by wt. In experiments with purée, the flesh from peeled and cored pears was rapidly blended in the above proportion with similarly prepared syrup to which 3% sodium chloride had been added. Processing was carried out in nylon pouches whenever it was necessary to ensure absence of tin ions from the control pack.

In experiments with chemical additives, the concentration of tin salts was expressed as ppm tin, and sulphur dioxide and hydrogen sulphide were added as sodium metabisulphite and sodium sulphide, respectively.

The colour-generating potential of pear material, i.e. the leucocyanidin number (*LN*), and the shade and depth of 'red' and 'purple' colours developed on processing (*x* and *y* values) were measured by methods already described.[2]

Effect of tin ions on developed and potential colour in heated pear purée

Packham pear purée (*LN* 236) was processed in nylon pouches for 1 h at 100° with the addition of varying amounts of stannous chloride. The control sample without tin turned a grey pink and samples with less than 50 ppm tin turned bright purple-pink; samples with more than 50 ppm tin retained their creamy yellow colour, and even on heating for 4 h were only discoloured a light brown. As previously observed,[2] there was an increase of *LN* on heating, but addition of tin ions reduced the colour potential of the heated purées, which reached a limiting value with the addition of 100 ppm tin (Fig. 1).

* Part II: Preceding paper.

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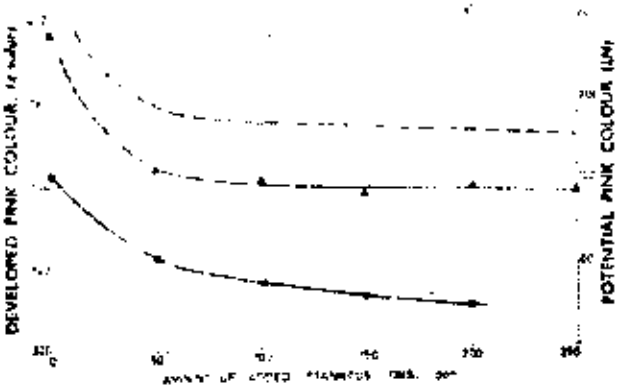


FIG. 1. *Potential and developed colour of pear purées heated with varying amounts of stannous ions*

▲ Potential colour in purée (*LN* 286) heated 1 h; ▲ developed colour in purée (*LN* 515) heated 2 h; ■ developed colour in purée (*LN* 382) heated 1 h

Two purées of *LN* 382 and 515 were heated for 1 and 2 h respectively with varying amounts of stannous chloride; the *x* values are recorded in Fig. 1. In the purée with the lower *LN*, pinking was inhibited at 50 ppm tin, and there were negligible differences in the *x* values (356 ± 2). In the other sample, pinking was reduced but the *x* values tended to remain constant after 100 ppm, while the *y* values (335 ± 3) of the tin-treated samples were lower than that of the control (351) but still not low enough for the purée to appear purple.[2] From similar experiments the efficiency of known levels of stannous ions in preventing colour development was found to depend on leucocyanidin content. Thus, 100 ppm tin prevented pigmentation in purées of *LN* = 350 heated for 2 h at 100° while 250 ppm controlled discoloration in similarly treated purées of *LN* = 750.

Packham pear purée was processed in nylon pouches at 100° for 1 h and re-packed into nylon pouches with and without the addition of stannous chloride before a second processing at 100° for 2 h. The heated purée was initially grey pink; the colour merely darkened slightly in the absence of tin, but intense purple-pink discolorations developed in the presence of tin at 50 ppm and at 100 ppm. When tin (100 ppm) was added to the purée before any heat treatment, discoloration was limited to the light buff shade of overprocessed pears.

Inhibition of colour formation in pear extractives by tin ions

A salt-free Packham pear purée which gave an immediate *LN* of 312 was treated with 25 and 50 ppm tin and allowed to stand for 4 h. The purées were then light brown and creamy yellow and gave *LN* of 260 and 286 respectively, while the untreated control was very dark brown and gave a *LN* of 148.

Determinations of *LN* were carried out on a Packham pear purée with combinations of three variations on the procedure: heating the purée prior to extraction with butanol, addition of tin to the purée, and addition of tin to the extract. A significant reduction in developed colour only occurred if heat was applied after the addition of tin (Table I).

TABLE I

Effect of adding tin at various stages in the determination of the potential colour (*LN*) of pear purées

Tin added to purée, ppm	Time of heating of purée prior to extraction with butanol, min	Reading for *LN*	
		Tin not added to butanol extract	Tin (500 ppm) added to butanol extract
0	0	212	116
100	0	197	
0	60	290	152
100	60	185	

Effect of tin ions on discoloration in processed pears

Packham pear quarters were processed in plain tinplate cans at 100° with and without the addition of 250 ppm tin; samples were withdrawn at 30 min intervals for determinations of *LN* and *x* and *y* values. In Fig. 2 these values are plotted against time of process for one representative experiment. The tin contents of the drained quarters from these packs increased with processing time from 6 to 14 ppm in the control samples and from 20 to 32 ppm in the tin-treated samples.

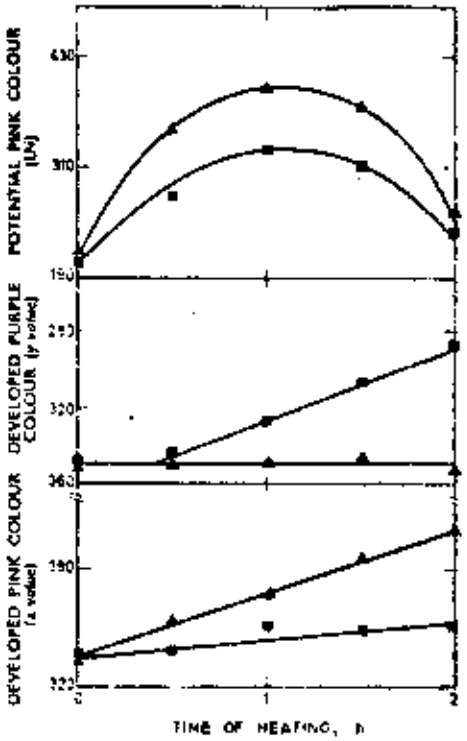


FIG. 2. *Potential and developed colour in pear quarters heated for varying times with and without stannous ions*

▲ No stannous ions added; ■ 250 ppm stannous ions added

Packham pear quarters were held 4 h in 3% sodium chloride at 20° with and without the addition of 200 ppm tin: the untreated samples were canned only in plain syrup, while the tin treated-samples were canned in both plain syrup and syrup containing 200 ppm tin. Samples were withdrawn after 30, 60, 90, and 120 min at 100° for visual comparison of colour. The discoloration in the control samples was marginally less than that in the tin-treated samples canned in either tin-free or tin-treated syrup. The tin contents of the drained halves from these packs increased with the processing time from 1 to 8 ppm, 31 to 43 ppm, and 56 to 60 ppm, respectively.

TABLE II

Effect of tin on discoloration of pears processed in nylon pouches

Pre-processing bath	Tin content of added syrup, ppm	Degree of discoloration	Tin content of quarters or purée, ppm
Pear quarters:			
3% brine	—	*	—
3% brine	30	**	20
3% brine + 300 ppm tin	—	***	32
300 ppm tin	—	****	27
300 ppm tin	100	****	63
Pear purée:			
300 ppm tin	—	†	67

† No discoloration
**** Extreme discoloration

Packham pear quarters were divided into three batches, held separately at 20° for 15 min with gentle agitation in baths of 3% brine, water containing 300 ppm tin, and 3% brine containing 300 ppm tin. The quarters were then processed as such or as purée in nylon pouches, with and without the addition of tin. After 2 h at 100° samples were examined for appearance and tin content; significant results only are shown in Table II.

Packham pear quarters were divided into two batches, one of which was subjected to ultrasonic vibrations for 30 min in 3% sodium chloride before both were gently agitated 15 min in a bath containing 300 ppm tin. When processed at 100° for 2 h in nylon pouches with syrup containing 100 ppm tin, the two batches showed extreme discoloration with negligible differences in shade or depth of colour or in the tin contents (69 ppm) of the drained halves.

Effect of various chemical additives on discoloration in pear purée

William B.C. pear purée (*LN* 181) was treated separately with stannous chloride, stannous citrate, stannic chloride, and aluminium chloride at a metallic ion concentration of 100 ppm, and with sulphur dioxide at 100 ppm. After processing for 150 min at 100° in nylon pouches, the sulphited sample remained creamy yellow (*x*, 355; *y*, 356), while that treated with aluminium chloride showed the greatest discoloration (*x*, 383; *y*, 369); intermediate results were obtained with stannous chloride and stannous citrate (*x*, 366; *y*, 379 for both) and with stannic chloride, cupric chloride, and no additive (*x*, 379; *y*, 372 for each).

The effect of four other reducing agents was tested in a similar experiment with Packham pear purée (*LN* 465). The products from treatments with 100 ppm of oxalic acid (*x*, 390; *y*, 357), and arsenious oxide (*x*, 395; *y*, 356) were only very slightly less discoloured than the control (*x*, 404; *y*, 344). Ascorbic acid inhibited discoloration at 200 ppm but only in samples heated less than 60 min, and eventually the discoloration was more pronounced than in the control (Fig. 3) even though analyses showed that the pack still contained 85% of its original ascorbic acid. Hydrogen sulphide prevented pink discoloration at 50 ppm (*x*, 360; *y*, 375) but the samples developed definite yellow discolorations, especially those with more than 100 ppm hydrogen sulphide, probably as the result of formation of molecular sulphur.

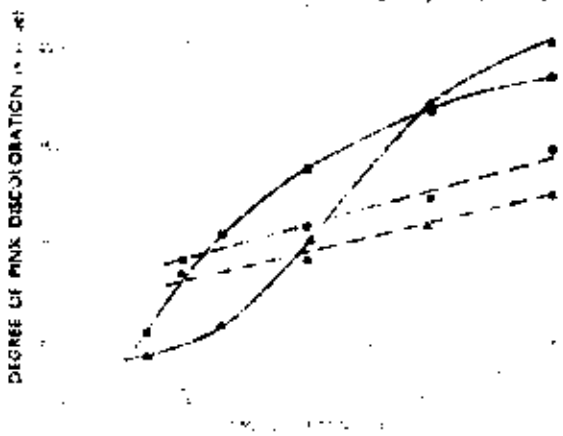


FIG. 3. *Effect of ascorbic acid and sulphur dioxide on discoloration in processed pears*

■ — ■ Pear purée heated without ascorbic acid;
▲ — ▲ Pear purée heated with ascorbic acid (200 ppm);
■ - - ■ Pear quarters heated without sulphur dioxide;
▲ - - - ▲ Pear quarters heated with sulphur dioxide (200 ppm)

Effect of decreasing headspace oxygen on discoloration in canned pears

The following procedures aimed at lowering the concentration of headspace oxygen were found to have no effect on the discoloration in canned Packham pears: sealing under high vacuum, flushing several times with nitrogen before sealing, and addition of oxidase and catalase enzyme systems with subsequent incubation for 4 h before processing. Analysis of the headspace gases in these packs showed that very low levels of residual oxygen had been achieved in some cases.

Inhibition of pear discoloration by sodium metabisulphite

Packham pear purée (*LN* 795) was processed at 100° in nylon pouches for 30, 60, and 120 min with and without the addition of 250 ppm sulphur dioxide to the syrup. The samples with sulphur dioxide showed no discoloration after 120 min, giving *x* and *y* values of 359 and 351, respectively; untreated samples showed a marked increase in *x* value from 329 to 363, 372, and 413 and a slight decrease in *y* value from 349 to 351, 347, and 343 over this period.

Packham pear purée (*LN* 660) was processed at 100° for 2 h in nylon pouches with the addition of varying amounts of sulphur dioxide to the syrup. The degree of pinking was measured by the *x* value with the results shown in Fig. 4; the samples showed no purpling, with little change in *y* value (350 ± 5).

Packham pear quarters were divided into three groups; one was processed without special treatment, another was canned in sulphited syrup (200 ppm sulphur dioxide), and the third was held in the sulphited syrup for 2 min at 100°, then drained and canned in normal syrup. The cans were processed at 100° and samples were withdrawn at 30 min intervals, the quarters were drained and were homogenised for examination. As expected, because the samples were packed in lacquered cans, discoloration was very slight and limited to processing times of 60 min and above; the discoloration was a grey pink with no purpling (*x* values 363 ± 5). In Fig. 3 the *x* values are plotted against processing time for the most and the least discoloured samples which were, respectively, the control sample and the sample canned in sulphited syrup. Sulphur

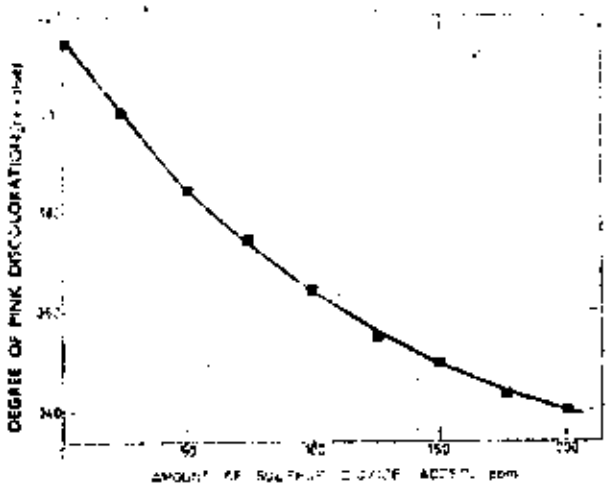


FIG. 4. *Discoloration in pear purée heated with varying amounts of sulphur dioxide*

dioxide was present at about 50 ppm in the samples canned in sulphited syrup, but could not be detected in the samples which had only been blanched in sulphited syrup.

Winter Cole pear halves showing pink discoloration (*x*, 425; *y*, 338) after processing in nylon pouches were repacked in nylon pouches with and without the addition of 500 ppm sulphur dioxide to the syrup. After 1 h at 100° the discoloration in the sulphited sample (*x*, 426; *y*, 349) differed in shade but not in total visual intensity from the halves heated without sulphite (*x*, 435; *y*, 335).

Six Packham pear purées of varying *LN* (296–795) were processed in nylon pouches for 2 h at 100° with the addition of 100, 125, 150, 175, 200, 225 and 250 ppm sulphur dioxide in the syrup. A highly significant correlation ($r = 0{\cdot}9944$) existed between the *LN* of the purée and the lowest concentration of sulphur dioxide (*SI*) required to prevent pink discoloration, according to the regression equation: $LN = 3{\cdot}32\,S - 32{\cdot}5$.

Organoleptic examination of pears canned with sulphur dioxide

Winter Cole pear quarters were separated into four batches, one-quarter to each batch, and held in 3% brine until processed. Two of the batches were canned in syrup to which 200 and 250 ppm sulphur dioxide had been added; these batches were filled into lacquered cans, while both plain tinplate and lacquered cans were used for the two unsulphited batches. The cans were sealed under 20 in. vacuum and processed for 60 min at 100°. The products were held at room temperature for 5 days and submitted for organoleptic examination to a panel of 63 experienced tasters who were asked to rank the four samples for colour, flavour, and general preference, and to comment on any off-flavours.

Statistical analysis of the taste test results (Table III) showed average treatment differences that were very highly significant for colour, highly significant for general preference, and not significant at $P = 0{\cdot}05$ for flavour. There were no significant differences between the controls processed in plain tinplate and lacquered cans, and significant differences only for colour between the two sulphited treatments. The sulphited pears were greatly preferred for their pale cream colour, their flavour was not impaired, and overall the sulphited samples were considered more acceptable than the untreated samples.

TABLE III

Mean ranks* recorded by a 63-member panel for pears canned with and without sulphur dioxide

	Plain tinplate cans without SO_2	Lacquered cans		
		Without SO_2	200 ppm SO_2	250 ppm SO_2
Colour	3·39[a]	3·34[b]	1·76	1·51
Flavour	2·47[b]	2·71[b]	2·52[b]	2·31[b]
General preference	2·70[c]	2·88[c]	2·31[d]	2·11[d]

* Lowest mean ranks correspond to most preferred product

a–d Any two figures not represented by the same letter are significantly different at the 5% level

Only five tasters reported off-flavours in the sulphited products that could be ascribed to the treatment, using the terms 'slight sulphur taste', 'as if sulphur dioxide present', 'sulphurous', 'sulphury', and 'sulphur dioxide'; only one taster noted an off-flavour that could be associated with sulphur dioxide in the product with the lower level of additive. Otherwise, comments on off-flavour were applied indiscriminately to sulphited and unsulphited samples. Analyses showed that the samples from the lower and the higher sulphite treatments contained 58 ppm and 75 ppm sulphur dioxide, respectively. Further storage for 12 months at room temperature did not affect the colour and flavour of either treated or untreated samples.

Discussion

A reduced level of 'pink' discoloration was previously reported[1] in heated purées to which tinplate discs had been added, the result of either an electrochemical reduction of the pigment by the dissolving metal or a reaction involving tin ions; the tin ions could have reduced the pigment or its precursors chemically, or prevented their production by complex formation. Experiments with stannous chloride as an additive showed that stannous ions were the active agent, inhibiting 'pink' discoloration in pear purées heated in nylon pouches, provided their concentration reached a critical level which was dependent on the leucocyanidin content of the purée. If the concentration of stannous ions was too low, the purple-pink tin complex was formed (Fig. 1).

Despite the results with pear purée, attempts to control the discoloration in canned pear halves by addition of stannous ions were entirely without success. Instead stannous ions produced an intensification of the discoloration in pear halves (Fig. 2), even at five times the concentration that prevented discoloration in comparable purées; the colour-generating potential of the treated halves had been lowered but not enough to prevent discoloration. Tin analyses indicated that inadequate penetration of stannous ions into pear halves could account for the difference in discoloration of halves and purée, but attempts to increase the penetration (by, for example, blanching baths and sonic treatment before processing) did not increase the tin contents enough to reduce the discoloration. Even when the final tin contents of the halves were the same as the final tin contents of comparable undiscoloured purée, the halves were intensely discoloured, suggesting that penetration had not been quick enough to interfere with colour development.

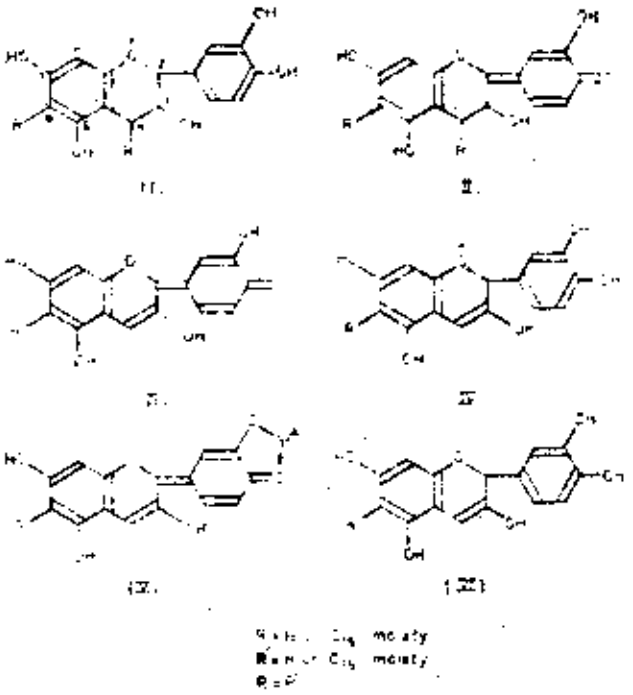
Replacement of stannous ions by a more efficient inhibitor of 'pink' discoloration required knowledge of the mechanism

of inhibition. Addition of stannous ions to discoloured puree demonstrated that their inhibition of discoloration was not due to chemical reduction of pigments already formed from leucocyanidin but to some reaction prior to pigment formation. Reactions of leucocyanidin with stannous ions seem limited to colourless salt formation since its currently accepted structure[3,4] contains no complex-forming group, the *o*-dihydroxy group in the *β*-ring having no chelating ability in non-ketonic flavonoids.[5] Catechin, with a structure very similar to leucocyanidin, has been reported[6] to form insoluble complexes with stannous ions, but, since no colour change is involved, the product is probably a simple insoluble salt similar to the soluble sodium and aluminium salts whose spectra also show negligible differences from that of catechin.[6,7] Such salts are unlikely to reduce discoloration in processed pear purées, and indeed it was found that aluminium ions produced intense discolorations and cupric ions had no effect whatever under conditions whereby stannous ions inhibited the discoloration.

Furthermore, addition of tin salts to pear purée did not significantly reduce its colour-generating potential unless heat was applied after the addition, whereas addition of tin salts greatly decreased the amount of pigment produced by extracted pear leucocyanidins, irrespective of whether or not the purée had been heated prior to extraction (Table I). These results may be explained by a reaction of stannous ions with an intermediate in the conversion of leucocyanidin to pigments, but not with leucocyanidin itself, and addition of various chemicals to purées processed in nylon pouches clearly demonstrated that the stannous ion behaves here as a reducing agent. Other strong reducing agents (sulphur dioxide, hydrogen sulphide, and ascorbic acid) were all effective in inhibiting discoloration, but not weaker reducing agents such as arsenious oxide and oxalic acid. A pre-processing bath with sulphur dioxide (3000 ppm) was part of a canning procedure previously suggested for certain pear varieties,[2] but its function was apparently to inhibit enzymic browning in the holding tanks since pink discoloration was still encountered.

Identification of a reduction as the reaction inhibiting pear discoloration conforms with the previous observation that leucocyanidin, which contains no readily reducible groups,[3,4] does not itself react with the inhibitor. Furthermore, it was shown that neither sulphur dioxide nor stannous chloride chemically reduce pigments already formed from degradation of leucocyanidin (I), but prevent or reverse this degradation by reducing intermediates in the breakdown. Quinone methines of the type II may be proposed as the intermediates concerned; they could be formed by enzymic or atmospheric oxidation of the leucocyanidin, they could be converted by heat via the anhydrobase (III) to cyanidin (IV), or in the presence of tin to the purple-pink tin complex (V), and their formation could be reversed or inhibited by the inhibitors of "pink" discoloration in processed pears. It may be noted that the suggested mechanism requires the positions of polymerisation of the leucocyanidin molecule to be the 4 and 6 (or 8) positions; if they were the only other presently accepted alternatives,[3,4] the 2 and the 6 (or 8) positions, the quinone methine structure could not be formed. These observations therefore have a bearing on this point of obscurity in leucocyanidin chemistry.

The first step in the process of "pink" discoloration is therefore seen as an oxidation of leucocyanidin (I) to the corresponding quinone methine (II) which may be reduced back to the *o*-dihydroxy compound (I). In the absence of reducing



agents, heat converts II into the conjugated anhydrobase (III) which would be a strong complexing agent for stannous ions, forming the purple-pink chelate (V), only one of whose many resonating structures is shown. In the absence of stannous ions, the anhydrobase would be converted principally to the colourless and unstable pseudobase (VI) in the weakly acidic conditions existing in canned pears, with smaller amounts of the pigment, cyanidin (IV), which requires pH levels below 3·0 for optimum stability.

Thus, whether stannous ions act to promote or inhibit discoloration in pears is determined not only by the amount of tin added but also by the time at which it becomes available to react with the products of leucocyanidin breakdown; if the degradation has proceeded only to the quinone methine (II), stannous ions will reverse the initial oxidation step, but if it has proceeded to the anhydrobase (III), stannous ions promote discoloration by the formation of the stable purple-pink tin complex (V). Stannous ions fail to inhibit discoloration in canned pears, but not in canned purée, because quinone methines are formed and degraded in pear halves before stannous ions sufficiently penetrate the tissue. Furthermore, this mechanism, which does not involve the formation of free cyanidin, accounts for discoloration occurring under conditions which normally lead to fading in cyanidin solutions and in foods containing low concentrations of cyanidin glycosides, such as certain cherry products.

Reducing agents are believed to improve the extraction of polyphenols from plant tissues by suppressing the oxidation to *o*-quinones, either by atmospheric or enzymic oxidation. For instance, addition of sulphur dioxide[8] increased the yield of total polyphenolics in persimmon extracts, but at the same time it decreased their conversion to pigments on heating with acid. Similarly, it has been found that stannous ions and sulphur dioxide, although inhibiting polyphenol oxidase in pear purées, lowered their colour generating potential. The suggestion is thus simply stated that reducing agents decrease

the pink discoloration in heated pears by reversing or inhibiting the oxidation of leucocyanidin to the quinone methine. This theory is not invalidated by discolorations in canned pears with reduced headspace oxygen, since oxidative reactions could still have occurred in the products.

To test the practical application of these findings, a pear variety highly susceptible to pink discoloration, the Winter Cole, was canned with and without the addition of sulphur dioxide. An alternative procedure using ascorbic acid may also have given satisfactory results, but it is a more expensive and less effective inhibitor of the discoloration than sulphur dioxide. The quality of the sulphited product was highly satisfactory; it had the attractive creamy-yellow colour normally associated with William B.C. pears processed in unlacquered cans and there was negligible off-flavour production, especially at the lower level of the additive (200 ppm). The concentration of residual sulphur dioxide in the processed product was low (less than 80 ppm) and did not present difficulties, except for prohibiting the use of unlacquered cans for the process. Of course, the use of lacquered cans would, by itself, obviate the problem of pink discoloration in William B.C. pears but a dull-looking product usually results. Because the presence of stannous ions normally enhances the colour of the product to a creamy yellow, only occasionally giving rise to pink discolorations when pears of abnormally high leucocyanidin content are canned the canned pear industry has been based on William B.C. pears, which have a low colour-generating ability,[2] processed in plain tinplate cans.

It is unlikely that the occasional occurrence of pink discoloration in canned William B.C. pears would justify a change to a sulphited pack in lacquered cans, even though the small amount of sulphur dioxide needed would lead to less than 40 ppm remaining in the product. However, if it is desired to can pear varieties now avoided because of their susceptibility to discoloration, such as Packhams, Winter Coles, Josephines, and Keiffers, a satisfactory process could be developed from these findings. Provided that food legislation permits, similar procedures using low levels of sulphur dioxide could also be used in the processing of other leucoanthocyanidin-containing foods subject to undesirable pink discoloration, such as lychees, gooseberries, okra, and chestnuts.

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ERRATA

In the paper by Bottrill & Hawker, *J. Sci. Fd Agric.*, 1970, **21**

Page 193, Table I, *for* pheopharbide *read* pheophorbide

Page 195, Fig. 4 caption *for* (a) *dipped and* (b) *undipped*
read (a) *undipped and* (b) *dipped*
In part (a)(iii) of the diagram the upper 'C' refers to 'C_a' and the lower 'c' to 'C_b'

Page 196, right-hand column, line 8, *for* Table II *read* Table III.

In the paper by Booth & Ewart, *J. Sci. Fd Agric.*, 1970, **21**

Page 188, Table I, bottom of Ponca γ_2 column *for* 10·5 *read* 1·05

bottom of Amino acid column should read $\frac{\text{Polar} + \text{ionic}}{\text{non-polar}}$

Committee on Specifications, 1972
Food Chemicals Codex
Committee on Food Protection, National Research
Council, National Academy of Sciences,
Washington, D. C.

AN IODOMETRIC METHOD FOR ASSAY OF TIN CONTENT

II. Modification of Iodometric Method for Assay of Tin Content

by

Bohdan Fitak and Andrzej Rajpert

From a research report on foodstuffs at the Academy of Medicine, Warsaw, Dr. S. Krauze, Dir.

A modification of the iodometric method for assay of tin content. Modification consists in lowering pH of reaction medium and increasing the amount of reducing agent for reduction of stannic salts to stannous salts.

The iodometric method is usually used to assay tin content in foodstuffs. The Polish Committee on Standards established the analytic standard for assay of tin content in nutritive substances (PN-59/A-04014) where the use of this method is recommended. This standard has been in effect in Poland for assaying tin content in foodstuffs since January 1, 1960. Nevertheless, the method recommended in the Polish Standards has provoked serious disagreements. It has been shown in a previous paper [1] that the iodometric method described in PN-59/A-04014, which has been verified in pure solutions, is burdened with significant control (negative) systematic error.

Findings obtained with the aid of this method produce a mean absolute error of about 50%, while the accuracy of the method is 2S = ±6.74% which excludes possible use of a correction factor. Thus, the method stated in the Polish Standards is not suitable for assaying tin content and must be replaced as quickly as possible.

Bearing in mind modest requirements for food control laboratory equipment, it was adopted to study the development of a modified iodometric method, permitting production of more detailed and accurate findings.

Experimental Part

Assays were carried out according to the method described in PN-59/A-04014 without allowing for the mineralization stage; the distinction was that 50ml HCl (1.19 density) and 12ml H_2SO_4 (1.84 density) were added to the solution of tin element, not 25ml HCl and 6ml H_2SO_4 as stated in the Standard. In addition, reduction of stannic salts to stannous salts was carried out using 0.6g of powdered Al from the Metallurgy plant of the "Halogen" Chemical Company, Warsaw, instead of 0.4g Al as the Standard states.

Reduction of stannic salts was carried out in a CO_2 atmosphere, using as carbon dioxide sources a Kipp generator (limestone and HCl 1 + 1 by weight) or a steel bottle of compressed CO_2.

When using a Kipp generator, the CO_2 is passed through three scrubbers containing, respectively: 5% solution of $CuSO_4$, 10% solution of $NaHCO_3$, and distilled water. When a CO_2 bottle is used, the carbon dioxide is passed through two scrubbers containing a solution of pyrogallol and distilled water.

The pyrogallol solution is prepared in the following manner:20g pyrogallol is dissolved in 50ml distilled water and is then mixed with a solution containing 180g potassium hydroxide in 300ml of distilled water.

Solutions in the scrubbers are changed after every five assays of tin content.

Simultaneously with the test sample, a determination of two or three solutions from a random sampling is carried out using the same method and same solutions (except for the tin element); from

the resultant findings, the mean arithmetic is produced and this is then subtracted from assay findings of samples containing tin.

A solution of tin element is used in assays which is prepared by dissolving granular tin waste (from FOCH Gliwica) in 100 ml of concentrated HCl(1.19 density) and subsequent addition of distilled water to make one liter. One ml of this solution contained about 0.5; 1.0; 1.2, or 1.5mg Sn.

For titration, titrated solutions of 0.01n I_2 and 0.01n $Na_2S_2O_3$ were used; titration was conducted daily [2]. Titration of 0.01n $Na_2S_2O_3$ is regulated with 0.01n basic potassium dichromate solution; titration of iodine - by 0.01n sodium thiosulphite solution.

The density of concentrated hydrochloric and sulphuric acids is verified against FP III [3].

Discussion of Findings

In the present paper, a modification of the iodometric method stated in the Polish Standards is described. The modification consists in lowering the pH of the medium by using an increased (doubled) amount of concentrated HCl and H_2SO_4 for reducing stannic salts to stannous salts. In addition, there is an increase in the quantity of reducing agent (powdered metallic Al) from 0.4 to 0.6g.

In developing the method, we avoid the mineralization stage and the possible losses coupled with it, based on the presumption that they are not the chief source of method error. Consequently, the present paper is concerned with correcting the last stage of this method, namely the reduction of stannic salts to stannous salts, the oxidization of Sn^{2+} using titrated iodine solution, and the titration of excess iodine with a titrated solution of sodium thiosulphite.

The resulting modified iodometric method is characterized by using statistical mathematical methods. For this purpose, a series of invariable assays is used to compute the accuracy and precision of the method.

Questionable findings have been produced conforming to modern criterion of deviation for questionable findings [4]:

$$t'_{\beta} \leq \frac{x_{n+1} - \bar{X}}{S}$$

where x_{n+1} - the questionable finding; $\bar{X}$ - the mean arithmetic of the series in question, without allowance for the questionable finding; S - the standard computed deviation for the series in question, without allowance for the questionable finding.

Twelve assays were performed in order to study the recovery of the modified iodometric method (using a Kipp generator) each having 5.09mg Sn per assay (cf. Table I, #4).

A mean recovery of 4.64mg Sn was produced, i.e., 91.17%. And thus, despite the production of a fairly high recovery, the method is burdened with systematic error (whose value is shown in Table 1, #4, col. 17: 4.91 > 3) which warrants the computation of a correction factor (k = 1.097). In return, the relative error of a single finding (V = 6.95%) and the detailed error of accuracy (B_d = 8.84%) are much less than in the

Table 1

Computed Characteristics of Test Findings on Assay of Tin Content Recovery in Basic Solution.

1	2	3	4	5	6	7	8	9	10
1	cylinder	12	10,14	85,93	— 14,07	4,50	18,86	20,24	12,14
2	" "	12	7,58	89,22	— 10,78	2,41	5,33	5,81	9,77
3	" "	11	6,17	93,57	— 6,43	1,74	2,74	3,01	5,99
4	Kipp generator	12	5,03	91,17	— 8,83	6,34	35,40	40,23	19,06
5	cylinder	12	3,09	96,33	— 3,67	2,0	3,55	3,98	5,7
6	" "	11	2,07	95,39	— 4,61	2,28	4,74	5,21	6,77
7	" "	12	1,098	98,11	— 1,89	2,71	6,88	7,51	8,39
8	" "	11	0,5489	99,09	— 0,91	2,66	6,43	7,07	8,72

1. Test number; 2. CO_2 source; 3. Number of assays n; 4. Quantity of Sn in Sample, mg.; 5. Mean Recovery x, %; 6. Mean absolute 100 - x, %; 7. Standard Deviation S [1], %; 8. Mean Deviation 0 [1], %; 9. Variance S [1], %; 10. Interval R [1], %.

Table 1, continued

Tabela 1 (c.d.)

L.p.	Średni rozstęp metody R w % [1]	Błąd standardowy Sx w % [1]	Względny błąd wyniku pojed. V w % [1]	Względny błąd wyniku średniego Vx w % [1]	Błąd dokładności B_d w % [1]	Przedział ufności dla poz. ufn. 0,95 $\bar{x} \pm t_\alpha^* \cdot S_{\bar{x}}$ w %	Badanie istotności błędu systemat. [1]	Mnożnik poprawkowy K [1]
	11	12	13	14	15	16	17	18
1	—	1,31	3,21	1,53	14,10	2,88	— 10,74	1,164
2	—	0,70	2,70	0,78	10,82	1,54	— 15,4	1,121
3	—	0,52	1,86	0,56	6,48	1,16	— 12,37	1,069
4	—	1,80	6,95	1,97	8,34	3,96	— 4,91	1,097
5	6,24	0,58	2,08	0,60	3,73	1,28	— 6,33	1,038
6		0,69	2,39	0,72	4,35	1,54	— 6,68	1,048
7	7,56	0,79	2,79	0,81	1,89	1,74	— 2,39	—
8		0,80	2,68	0,81	0,89	1,78	— 1,14	—

11. Mean method interval R, % [1]; 12. Standard error Sx, % [1]; 13. Relative error of single finding V, % [1]; 14. Relative error of average finding Vx, % [1]; 15. Error in accuracy B_d, % [1]; 16. Confidence limit for pos. conf. 0.95 $\bar{x} \pm t_\alpha^* \cdot S_{\bar{x}}$, %; 17. Study of significance of systematic error [1]; 18. Correction factor K [1].

(* taken from Fisher's table [4]).

Table 2

Computed Characteristics of Research into Determining Accuracy of Tin Content Assays in a Basic Tin Solution.

L.p.	Źródło CO_2	Ilość oznaczeń n	Ilość roztw. podst. Sn w próbce w ml	Ilość Sn w próbce w mg	Średni odzysk $\bar{x}$ w %	Odchylenie standardowe S w %	Precyzja 2S w %	Współczynnik precyzji h [1]
1	2	3	4	5	6	7	8	9
1	Kipp generator	35	4,93	4,99	91,48	3,76	7,52	0,19
2	cylinder	24	2,05 5,03	2,07 5,09	93,19	1,76	3,52	0,40
3	cylinder	25	4,93	4,99	94,78	1,14	2,28	0,62

1. Test number; 2. CO_2 source; 3. Number of assays n; 4. Amonnt of dissolved basic tin in sample, ml; 5. Amount of tin in sample, mg; 6. Mean recovery $\bar{x}$, %; 7. Standard Deviation S, %; 8. Accuracy 2S, %; 9. Accuracy factor h [1].

method conforming to Polish Standards [1].

Then, to determine the accuracy of the modified iodometric method (using a Kipp generator), thirty-seven assays were carried out (two findings rejected, 35 findings used for computation), each having 4.93ml of solution of tin element constituting 4.99mg Sn (Table 2). Mean deviation S = 3.76 and accuracy 2S = ±7.52% were produced.

It has been determined that in carrying out one assay the greatest error may constitute ±7.52% and similarly, in two simultaneous assays, ±15.04%. Assay accuracy of the average of two or more assays constitutes:

for 2 assays, $\bar{y} = \frac{7.52}{\sqrt{2}} = \frac{7.52}{1.4142} = 5.32\%$

for 3 assays, $\bar{y} = \frac{7.52}{\sqrt{3}} = \frac{7.52}{1.7321} = 4.34\%$

for 4 assays, $\bar{y} = \frac{7.52}{\sqrt{4}} = \frac{7.52}{2} = 3.76\%$

for 5 assays, $\bar{y} = \frac{7.52}{\sqrt{5}} = \frac{7.52}{2.2361} = 3.36\%$

Then the average of two parallel assays can be burdened with an error of ±5.32% while from 5 assays, according-

ly, ±3.36%.

It follows from Table 2 that the modified iodometric method when using a Kipp generator as the CO_2 source, in comparison with the method described in the Polish Standards [1], is extremely accurate; this is probably due to the use of a modification which is also more or less of the same degree of accuracy as undoubtedly results from the use of a Kipp generator as the CO_2 source.

For the same reason, findings produced via the modified iodometric method, in spite of the fact that they are extremely proximate to actual tin content in a basic solution and in spite of possible use of a correction factor (1.097), are unreliable in view of the large interval which characterizes the method.

The confidence limit of the method according to Polish Standards constitutes $\bar{x}$±4.32% [1]. The confidence limit for the moidfied method (Kipp generator), however, constitutes $\bar{x}$±3.96% (Table 1, #4, col. 16).

It is evident that in this situation attention ion has been focused at eliminating the Kipp gener-

ator and replacing it with another CO_2 source. Thus, the Kipp generator is replaced by a steel cylinder containing CO_2. Then, for the sake of determining recovery of the modified iodometric method (using a CO_2 cylinder), seven test series were conducted with different quantities of tin in the sample: from 10.14 to 0.5489mg Sn (Table 1).

As follows from table 1, col. 17, in the range of concentrations from 2-10mg Sn in a sample, the method is burdened with systematic error and thus, necessitates computation of a correction factor (table 1, col. 18).

For a content of 10.14mg Sn in a sample, a mean recovery of 85.93% was produced; for a content of 7.58mg Sn - 89.22%.

These recoveries, in comparison with subsequent series (table 1, #3, 5, 6, 7, and 8) and even in comparison with the modified method using a Kipp generator (table 1, #4) are highly underestimated. Thus, the errors of this series of assays (table 1, #1, 2) are exaggerated in comparison with #3, 5, 6, 7, and 8 of the same table.

We should therefore conclude that the quantity of tin in the sample (10.14mg and 7.58mg) is as great as the error source and can not be used.

Subsequently, for a content of 6.17mg Sn in the sample, 93.57% recovery was obtained. This recovery is equivalent to a recovery of 95.39% (table 1, #6) for a content of 2.07mg Sn in the sample, using the following model t of Student for a series having an identical quantity n [5]:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{S_x^2 + S_y^2}} ,$$

where t - the Student number; $\bar{x}$ - mean recovery of series x; $\bar{y}$ - mean recovery of series y; $S_{\bar{x}}$ - standard error of series x; $S_{\bar{y}}$ - standard error of series y.

A value of $t = 2.105 > t_{\alpha} = 0.05 = 2.086$ was obtained for n = 20.

The value t is subtracted from the table of critical values of t [4].

Consequently, the differences are substantial and thus, the amount of tin in the sample (6.17mg) is

as great as the source of error and can not be used. In a subsequent sequence of assays on the recovery rate, two assay series were conducted: with 5.09mg Sn in the sample having a recovery rate of 96.33%; and with 2.07 mg Sn in the sample having a recovery rate of 95.39%.

These recoveries are equivalent to using the Student test for series having different degrees of freedom, using the following model [6]:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\sum(x_i - \bar{x})^2 + \sum(y_i - \bar{y})^2}} \cdot \sqrt{\frac{n_x \cdot n_y (n_x + n_y - 2)}{n_x + n_y}}$$

where t - the Student number; $\bar{x}$ - mean recovery of series x; $\bar{y}$ - mean recovery of series y; x_i - partial result in series x; y_i - partial result in series y; n_x - number of assays in series x; n_y - number of assays in series y.

A value of $t = 1.042 < t_{\alpha} = 0.05 = 2.080$ was obtained for n = 21.

The differences are not substantial and thus, a basis is obtained for computing the method's mean correction factor in concentration limits from 2-5mg Sn in the sample which constitutes 1.043. The mean recovery of the method constitutes 95.86%, the mean

interval - 6.24%.

For larger amounts of tin in the sample (about 1 and 0.5mg), recovery rates of 98.11 and 99.09% were obtained. For these amounts of tin the method is not burdened with systematic error (table 1., col. 17, #7 and 8), however it reveals an increased value in standard error as well as in the relative error of a single finding and the mean finding. In addition, the confidence limit range increases: for an amount of about 0.5mg Sn in a sample it reaches a value of $\bar{x}$±1.78%. A recovery of 98.11% is equivalent to the recovery obtained for 2mg Sn in a sample (95.39%). A value of $t = 2.572 > t_{\alpha} = 0.05 = 2.080$ for $n = 21$ was obtained. Thus, differences are substantial.

Summing up, the highest tin content recovery in a basic solution is obtained by the modified iodometric method using a cylinder as CO_2 source, as well as an amount of Sn per sample from 0.5 to 5mg. It has been found, however, that the best results can be obtained using the aforementioned method for content in the range 2-5mg Sn per sample (table 1, #5 & 6) with the mean correction factor.

Studying the accuracy of the modified iodometric method when utilizing a cylinder CO_2 source has confirmed that the method's precision depends, to a great extent, on the amount of tin in the sample (from about 2mg to about 5mg Sn). Twenty-five assays of tin content in a basic Sn solution were then carried out (one finding rejected and thus, for subsequent computations 24 findings were used), each assay having 2.05ml of basic solution, constituting 2.07mg Sn (table 2, #2).

A standard deviation S = 1.76 and precision 2S = = ±3.52% were produced.

This signifies that in conducting one assay, the greatest error may constitutes 3.52% and accordingly, ±7.04% for two parallel assays.

Thus, the specification accuracy of the average of two or more assays constitutes:

$$\text{for 2 assays, } \bar{y} = \frac{3.52}{\sqrt{2}} = \frac{3.52}{1.4142} = 2.49\%$$

$$\text{for 3 assays, } \bar{y} = \frac{3.52}{\sqrt{3}} = \frac{3.52}{1.7321} = 2.03\%$$

for 4 assays, $\bar{y} = \frac{3.52}{\sqrt{4}} = \frac{3.52}{2} = 1.76\%$

for 5 assays, $\bar{y} = \frac{3.52}{\sqrt{5}} = \frac{3.52}{2.2361} = 1.57\%$

Consequently, the average of two parallel assays can be burdened with an error of ±2.49%, whereas from five assays - ±1.57%.

Twenty-five assays of tin content in a basic solution were subsequently completed, each assay having 5.03 and subsequently, 4.93ml of basic solution, which comprises 5.09 and 4.99mg Sn (table 2, #3).

A standard deviation S = 1.14 and precision 2S = = 2.28% were produced.

This signifies that in conducting a single assay, the greatest error may constitute ±2.28% and consequently, for two parallel assays ±4.56%.

Thus, specification accuracy of the average of two or more assays constitutes:

for 2 assays, $\bar{y} = \frac{2.28}{\sqrt{2}} = \frac{2.28}{1.4142} = 1.61\%$

$$\text{for 3 assays, } \bar{y} = \frac{2.28}{\sqrt{3}} = \frac{2.28}{1.7321} = 1.32\%$$

$$\text{for 4 assays, } \bar{y} = \frac{2.28}{\sqrt{4}} = \frac{2.28}{2} = 1.14\%$$

$$\text{for 5 assays, } \bar{y} = \frac{2.28}{\sqrt{5}} = \frac{2.28}{2.2361} = 1.02\%$$

Thus, the average of two parallel assays can be burdened with an error of ±1.61%, whereas from five assays ±1.02%.

It was then verified whether or not the precision 2S= ±3.51 accordingly diverges from precision 2S = ±2.28.

To verify the significance of the differences between precisions, the Snedecor F test was used [7]:

$$F = \frac{S_{\bar{x}_1}^{\ 2}}{S_{\bar{x}_2}^{\ 2}},$$

where F- the Snedecor numbers; $S_{\bar{x}_1}^{\ 2}$ - variance of the mean arithmetic of one series; $S_{\bar{x}_2}^{\ 2}$ - variance of the mean arithmetic of the other series.

Variance of the mean arithmetic is computed according to the following model [7]:

$$S_{\bar{x}}^2 = \frac{S^2}{n}$$

where $S_{\bar{x}}^2$ - variance of mean arithmetic; S^2 - test variance; n - number of assays.

A value $F = 2.45 > F_{\alpha = 0.05} = 1.96$ is produced for n = 24 and 25. Thus, $F = 2.45 < F_{\alpha = 0.01} = 2.62$ for n = 24 and 25.

The following three instances may arise in a significance test:

(1) the value F computed on the basis of assay results is less than the value F read from the tables at a significance level of 0.05. Assays are identically precise;

(2) the value F from testing is greater than the value F from the tables at a significance level of 0.05 and less than the value F from the tables at a significance level of 0.01. Assays can be interpreted as roughly identical in precision;

(3) the value F from testing is greater than the value F from the tables at a significance level 0.01. Assays conducted are of different precisions.

And thus, in the aforementioned instance, differences between test precisions are not substantial and assays were conducted at approximately the same precision. We can therefore conclude that precision of the modified iodometric method using a cylinder CO_2 source does not depend on the amount of tin within the concentration range from about 2 to about 5mg Sn in the sample.

Conclusions

1. The modified iodometric method for the assay of tin content which was discussed required the production of corrected findings using a correction factor. Mean recovery of 95.86% and mean accuracy value of 2S = 2.9% were produced for this method. Modification of the method consists in lowering the pH of the reaction medium as well as increasing the amount of reducing agent used for reduction of stannic salts to stannous salts.

2. When using the modified iodometric method, we should avoid using a Kipp generator as the CO_2 source. Good results have been produced by the use of a compressed CO_2 cylinder for that purpose.

3. The iodometric method modification discussed required the production of corrected findings solely

within the concentration range from 2-5mg Sn per sample.

4. The modified iodometric method for assay of tin content is burdened with a small control (negative) systematic error. The computed mean value of the correction factor for concentrations from 2-5mg Sn per sample is 1.043.

5. The modified iodometric method using a correction factor can be introduced as a compulsory method for the assay of tin content in foodstuffs, instead of the currently used method per PN-59/A-04014.

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IODOMETRIC METHOD FOR ASSAYING TIN CONTENT (82)

II MODIFICATION TO IOD. METH FOR ASSAYING TIN CONTENT

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METODA JODOMETRYCZNA OZNACZANIA ZAWARTOŚCI CYNY

CZ. II. MODYFIKACJA JODOMETRYCZNEJ METODY OZNACZANIA ZAWARTOŚCI CYNY

Z Zakładu Badania Środków Spożywczych Akademii Medycznej w Warszawie
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Opracowano modyfikację jodometrycznej metody oznaczania zawartości cyny. Modyfikacja ta polega na obniżeniu pH środowiska reakcji i zwiększeniu ilości środka redukującego sole cynowe do soli cynawych. Podano charakterystykę statystyczną opracowanej metody.

Metoda jodometryczna jest powszechnie stosowana do oznaczania zawartości cyny w artykułach żywności. Polski Komitet Normalizacyjny opracował normę analityczną oznaczania zawartości cyny w środkach spożywczych (PN-59/A-04014), w której zaleca stosowanie tej metody. Norma ta jest obowiązująca w Polsce do oznaczania zawartości cyny w artykułach żywności od 1. I. 1960 r. Jednakże metoda zalecana w PN budzi poważne zastrzeżenia. W poprzedniej pracy [1] wykazano, że metoda jodometryczna opisana w PN-59/A-04014, którą sprawdzono na roztworach czystych jest obarczona dużym kierunkowym (ujemnym) błędem systematycznym.

Wyniki otrzymane za jej pomocą wykazują średni błąd bezwzględny około 50%, zaś precyzja metody 2S = ± 6,74% co wyklucza ewentualne zastosowanie mnożnika poprawkowego. A zatem metoda podana w PN nie nadaje się do oznaczania zawartości cyny i powinna być jak najszybciej wycofana.

Biorąc pod uwagę zapotrzebowanie skromnie wyposażonych laboratoriów kontroli żywności, podjęto prace nad opracowaniem modyfikacji metody jodometrycznej, pozwalającej na otrzymywanie bardziej dokładnych i precyzyjnych wyników.

CZĘŚĆ DOŚWIADCZALNA

Oznaczenia przeprowadzono według metody opisanej w PN-59/A-04014 pomijając etap mineralizacji z tą różnicą, że do roztworu podstawowego cyny dodawano 50 ml HCl o gęstości 1,19 i 12 ml H_2SO_4 o gęstości 1,84 a nie, jak podaje norma, 25 ml HCl i 6 ml H_2SO_4. Ponadto redukcję soli cynowych do cynawych przeprowadzano za pomocą 0,6 g sproszkowanego glinu metalicznego prod. Zakładów Chemicznych „Halogen" Warszawa, zamiast, jak podaje norma, 0,4 g glinu.

Redukcję soli cynowych przeprowadzono w atmosferze CO_2 stosując do otrzymywania dwutlenku węgla aparat *Kippa* (marmur i kwas solny 1+1 wagowo) lub butlę stalową ze sprężonym dwutlenkiem węgla.

W przypadku aparatu *Kippa* CO_2 przepuszczano przez 3 płuczki zawierające kolejno: 5%-owy roztwór $CuSO_4$, 10%-owy roztwór $NaHCO_3$ i wodę destylowaną.

W przypadku zaś butli z dwutlenkiem węgla, CO_2 przepuszczano przez 2 płuczki zawierające: roztwór pirogalolu i wodę destylowaną.

Roztwór pirogalolu przygotowywano w następujący sposób: 20 g pirogalolu cz. rozpuszczano w 50 ml wody destylowanej i mieszano z roztworem zawierającym 180 g wodorotlenku potasowego czda rozpuszczonego w 300 ml wody destylowanej.

Roztwory w płuczkach zmieniano zawsze po wykonaniu 5 oznaczeń zawartości cyny.

Równolegle z badaną próbką wykonywano w ten sam sposób, z tymi samymi roztworami (za wyjątkiem roztworu podstawowego cyny) oznaczenia w 2 lub 3 roztworach ślepej próby i z otrzymanych wyników obliczano średnią arytmetyczną, którą odejmowano od wyników oznaczeń prób zawierających cynę.

Do oznaczeń używano roztworu podstawowego cyny przygotowanego przez rozpuszczenie cyny granulowanej czda prod. FOCH Gliwice w 100 ml stęż. kwasu solnego o gęstości 1.19, a następnie dopełnienie wodą destylowaną do objętości 1 l. 1 ml tego roztworu zawierał około 0.5; 1.0; 1.2 lub 1.5 mg Sn.

Do miareczkowania używano miianowane roztwory 0.01n J_2 i 0.01n $Na_2S_2O_3$, których miano sprawdzano [2] codziennie. Miano 0.01n $Na_2S_2O_3$ nastawiano na 0.01n roztwór podstawowy dwuchromianu potasowego, zaś miano jodu na 0.01n roztwór tiosiarczanu sodowego.

Gęstość stężonych kwasów solnego i siarkowego sprawdzano wg FP III [3].

OMÓWIENIE WYNIKÓW

W niniejszej pracy opisano modyfikację metody jodometrycznej podanej w PN. Modyfikacja ta polegała na obniżeniu pH środowiska poprzez zastosowanie zwiększonej (podwójnej) ilości stężonych kwasów HCl i H_2SO_4 do redukcji soli cynowych do cynawych. Poza tym zwiększono również ilość środka redukującego (sproszkowanego, metalicznego glinu) z 0,4 do 0.6 g.

W opracowaniu metody pominięto etap mineralizacji i straty ewentualnie z nią związane, wychodząc z założenia, że nie one są zasadniczym źródłem błędów metody. A zatem niniejsza praca obejmuje skorygowanie ostatniego stadium metody, a więc redukcję soli cynowych do cynawych, utlenienie Sn^{2+} za pomocą miianowanego roztworu jodu i odmiareczkowanie nadmiaru jodu miianowanym roztworem tiosiarczanu sodowego.

Opracowaną modyfikację metody jodometrycznej scharakteryzowano za pomocą metod statystyki matematycznej. W tym celu wykonano szereg oznaczeń koniecznych dla obliczenia dokładności i precyzji metody.

Wyniki wątpliwe sprawdzano według nowoczesnego kryterium odrzucania wyników wątpliwych [4]:

$$r_5 = \frac{X_{n+1} - \bar{X}}{S},$$

w którym:

X_{n+1} — wynik wątpliwy,

$\bar{X}$ — średnia arytmetyczna badanego szeregu bez uwzględnienia wyniku wątpliwego,

S — odchylenie standardowe obliczone dla badanego szeregu bez uwzględnienia wyniku wątpliwego.

Badając odzysk zmodyfikowanej metody jodometrycznej (przy zastosowaniu aparatu *Kippa*) wykonano 12 oznaczeń (tab. I, poz. 4) biorąc do

Tabela I

Liczby charakterystyczne wyników badań nad ustaleniem odzysku zawartości cyny w roztworze podstawowym.

L. p.	Źródło CO_2	Ilość oznaczeń n	Ilość Sn w próbce w mg	Średni odzysk x w %	Średni bezwzgl. 100 − x w %	Odchylenie standard. S [1] w %	Odchylenie przeciętne δ [1] w %	Wariancja S^2 [1] w %	Rozstęp R [1] w %
1	2	3	4	5	6	7	8	9	10
1	butla stalowa	12	10,14	85,93	— 14,07	4,50	18,86	20,24	12,14
2	" "	12	7,58	89,22	— 10,78	2,41	5,33	5,81	9,77
3	" "	11	6,17	93,57	— 6,43	1,74	2,74	3,01	5,99
4	Aparat Kippa	11	5,09	91,17	— 8,83	6,34	35,46	40,23	18,96
5	butla stalowa	12	5,09	96,33	— 3,67	2,0	3,65	3,98	5,7
6	" "	11	2,07	95,39	— 4,61	2,28	4,74	5,21	6,77
7	" "	12	1,098	98,11	— 1,89	2,74	6,88	7,51	8,39
8	" "	11	0,5489	99,09	— 0,91	2,06	6,43	7,07	6,12

Tabela I (c. d.)

L. p.	Średni rozstęp metody R w % [1]	Błąd standardowy Sx w % [1]	Względny błąd wyniku pojed. V w % [1]	Względny błąd wyniku średniego Vx w % [1]	Błąd dokładności B_d w % [1]	Przedział ufności dla poz. ufn. 0,95 $\bar{x} \pm t_{\alpha} \cdot Sx$ w %	Badanie istotności błędu systemat. [1]	Mnożnik poprawkowy K [1]
	11	12	13	14	15	16	17	18
1	—	1,31	5,71	1,53	14,10	2,69	— 10,74	1,164
2	—	0,79	2,70	0,79	10,82	1,51	— 15,4	1,121

*) odczytano z tab. *Fishera* [4]

	11	12	13	14	15	16	17	18
3	—	0,52	1,86	0,56	6,48	1,16	— 12,37	1,069
4	—	1,80	6,95	1,07	8,84	3,96	— 4,91	1,097
5	6,24	0,58	2,09	0,60	3,73	1,28	— 6,33	1,036
6		0,69	2,39	0,72	4,35	1,54	— 6,68	1,048
7	7,56	0,79	2,79	0,81	1,89	1,74	— 2,39	—
8		0,80	2,68	0,81	0,89	1,78	— 1,14	—

Tabela II

Liczby charakterystyczne wyników badań nad ustaleniem precyzji oznaczeń zawartości cyny w roztworze podstawowym cyny.

L.p.	Źródło CO_2	Ilość oznaczeń n	Ilość roztw. podst. Sn w próbce w ml	Ilość Sn w próbce w mg	Średni odzysk $\bar{x}$ w %	Odchylenie standardowe S w %	Precyzja 2S w %	Współczynnik precyzji h [1]
1	2	3	4	5	6	7	8	9
1	Aparat Kippa	35	4,93	4,99	91,46	3,76	7,52	0,19
2	butla stalowa	24	2,05 5,03	2,07 5,09	93,19	1,76	3,52	0,40
3	butla stalowa	25	4,93	4,99	94,78	1,14	2,28	0,62

każdego oznaczenia po 5.09 mg Sn. Uzyskano średni odzysk 4,64 mg Sn tj. 91,17%. A więc metoda ta pomimo otrzymania dość dużego odzysku jest obarczona błędem systematycznym (wartość podana w tab. I, poz. 4, kol. 17—4,91 > 3), co upoważnia do obliczenia mnożnika poprawkowego (K = 1,097). Natomiast względny błąd wyniku pojedynczego (V = 6,95%), względny błąd wyniku średniego ($V_{\bar{x}}$ = 1,97%), a szczególnie błąd dokładności (B_d = 5,84%) są dużo mniejsze niż w metodzie według PN [1].

Następnie dla ustalenia precyzji zmodyfikowanej metody jodometrycznej (przy zastosowaniu aparatu *Kippa*) wykonano 37 oznaczeń (dwa wyniki odrzucono, a więc do obliczeń wzięto 35 wyników) biorąc do każdego po 4,93 ml roztworu podstawowego cyny co stanowi 4,99 mg Sn (tab. II). Uzyskano odchylenie średnie S = 3,76 i precyzję 2 S = ±7,52%.

Oznacza to, że przy wykonaniu jednego oznaczenia największy błąd może wynosić ±7,52%, zaś analogicznie przy dwóch równoległych oznaczeniach ±15,04%. Dokładność wyznaczania średniej z dwóch i więcej oznaczeń wynosi:

dla 2 oznaczeń $\bar{y} = \frac{7,52}{\sqrt{2}} = \frac{7,52}{1,4142} = 5,32\%$

" 3 " $\bar{y} = \frac{7,52}{\sqrt{3}} = \frac{7,52}{1,7321} = 4,34\%$

" 4 " $\bar{y} = \frac{7,52}{\sqrt{4}} = \frac{7,52}{2} = 3,76\%$

" 5 " $\bar{y} = \frac{7,52}{\sqrt{5}} = \frac{7,52}{2,2361} = 3,36\%$

Zatem średnia z dwóch równoległych oznaczeń może być obarczona błędem ±5,32%, zaś odpowiednio z 5 oznaczeń ±3,36%.

Z tabeli II wynika, że zmodyfikowana metoda jodometryczna przy zastosowaniu aparatu *Kippa* jako źródła CO_2 w porównaniu z metodą opisaną w PN [1] jest bardziej dokładna, co prawdopodobnie jest spowodowane zastosowaniem modyfikacji oraz mniej więcej w tym samym stopniu precyzyjna, co niewątpliwie wynika z zastosowania aparatu *Kippa* jako źródła dwutlenku węgla.

Dlatego też wyniki otrzymywane za pomocą zmodyfikowanej metody jodometrycznej (przy zastosowaniu aparatu *Kippa*) pomimo tego, że są bardzo zbliżone do prawdziwej zawartości cyny w roztworze podstawowym oraz pomimo ewentualnego zastosowania współczynnika korekcyjnego (1,097) są niepewne ze względu na duży rozstęp charakteryzujący tę metodę.

Przedział ufności dla metody według PN wynosi $\bar{x} \pm 4,32\%$ [1]. Zaś przedział ufności dla zmodyfikowanej metody (aparat Kippa) wynosi $\bar{x} \pm 3,96\%$ (tab. I, poz. 4, kol. 16).

Jest oczywiste, że w tej sytuacji skierowano uwagę na wyrugowanie aparatu *Kippa* i zastąpienie go innym przyrządem będącym źródłem CO_2. Aparat *Kippa* zastąpiono więc butlą stalową z dwutlenkiem węgla. Następnie w celu ustalenia odzysku zmodyfikowanej metody jodometrycznej (przy zastosowaniu butli stalowej z CO_2) wykonano 7 serii badań z różnymi ilościami cyny w próbce: od 10,14 do 0,5469 mg Sn (tab. I).

Jak wynika z tab. I, kol. 17 w zakresie stężeń od 2—10 mg Sn w próbce metoda jest obarczona błędem systematycznym, a więc jest podstawa do obliczenia mnożnika poprawkowego (tab. I, kol. 18).

Przy zawartości 10,14 mg Sn w próbce uzyskano średni odzysk 85,93% a przy zawartości 7,58 mg Sn — 89,22%.

Odzyski te w porównaniu z następnymi seriami (tab. I, poz. 3, 5, 6, 7 i 3) a nawet w porównaniu ze zmodyfikowaną metodą przy zastosowaniu aparatu *Kippa* (tab. I, poz. 4) są mocno zaniżone. Zaś błędy tych serii oznaczeń (tab. I, poz. 1 i 2) w porównaniu z poz. 3, 5, 6, 7 i 8 w tej tabeli są zwiększone.

A zatem należy wyprowadzić wniosek, że ta ilość cyny w próbce (10,14 mg i 7,58 mg) jest za duża, jest źródłem błędów i nie powinna być stosowana.

Następnie przy zawartości 6,17 mg Sn w próbce otrzymano 93,57% odzysku. Odzysk ten porównano z odzyskiem przy zawartości 2,07 mg Sn w próbce — 95,39% (tab. I, poz. 6) za pomocą następującego wzoru t *Studenta* dla szeregów o jednakowej ilości n [5]:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{S_x^2 + S_y^2}},$$

w którym:

t — liczba Studenta,
$\bar{x}$ — średni odzysk szeregu x,
$\bar{y}$ — średni odzysk szeregu y,
S_x — błąd standardowy szeregu x,
S_y — błąd standardowy szeregu y.

Otrzymano wartości $t = 2{,}105 > t_{\alpha=0{,}05} = 2{,}086$ dla $n = 20$.

Wartość t_α odczytano z tab. wartości krytycznych t [4].

Różnice są więc istotne, a zatem ta ilość cyny w próbce (6,17 mg) jest również za duża, jest źródłem błędów i nie powinna być stosowana. W dalszym ciągu badań wielkości odzysków wykonano 2 serie oznaczeń: z ilością 5,09 mg Sn w próbce, dla której otrzymano odzysk 96,33% i z ilością 2,07 mg Sn w próbce, dla której otrzymano odzysk 95,39%.

Odzyski te porównano za pomocą testu *Studenta* dla szeregów o różnej ilości stopni swobody stosując następujący wzór [6]:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\sum(x_i - \bar{x})^2 + \sum(y_i - \bar{y})^2}} \cdot \sqrt{\frac{n_x \cdot n_y (n_x + n_y - 2)}{n_x + n_y}}$$

w którym:

t — liczba *Studenta*,
$\bar{x}$ — średni odzysk szeregu x,
$\bar{y}$ — średni odzysk szeregu y,
x_i — poszczególny wynik w szeregu x,
y_i — poszczególny wynik w szeregu y,
n_x — ilość oznaczeń w szeregu x,
n_y — ilość oznaczeń w szeregu y.

[illegible] Średni odzysk metody wynosi 95,86% zaś średni rozstęp — 6,24%.

Dla mniejszych ilości cyny w próbce (około 1 i 0,5 mg) otrzymano odzyski 98,11 i 99,09%. Dla tych ilości cyny metoda nie jest obarczona błędem systematycznym (tab. I, kol. 17, poz. 7 i 8), jednak wykazuje wzrost wartości błędu standardowego oraz względnego błędu wyniku pojedynczego i wyniku średniego. Również zakres przedziału ufności rośnie i dla ilości ok. 0,5 mg Sn w próbce osiąga wartość $\bar{x} \pm 1,78\%$. Odzysk 98,11% porównano z odzyskiem uzyskanym dla 2 mg Sn w próbce (95,39%). Otrzymano wartość $t = 2,572 > t_{\alpha = 0,05} = 2,080$ dla $n = 21$. Różnice są więc istotne.

Reasumując, najwyższe wartości odzysku zawartości cyny w roztworze podstawowym cyny daje zmodyfikowana metoda jodometryczna przy zastosowaniu butli stalowej jako źródła dwutlenku węgla oraz przy ilości od 0,5 do 5 mg Sn w próbce. Jednak wydaje się, że najlepsze wyniki można otrzymać powyższą metodą przy zawartości w granicach 2—5 mg Sn w próbce (tab. I, poz. 5 i 6), stosując średni mnożnik poprawkowy.

Badając precyzję zmodyfikowanej metody jodometrycznej przy użyciu butli stalowej jako źródła CO_2 wykazano, że wielkość precyzji tej metody nie zależy w istotnym stopniu od ilości cyny w próbce (w granicach od około 2 mg do ok. 5 mg Sn). Wykonano więc 25 oznaczeń zawartości cyny w roztworze podstawowym Sn (jeden wynik odrzucono, a więc do dalszych obliczeń wzięto 24 wyniki), biorąc do każdego oznaczenia po 2,05 ml roztworu podstawowego co stanowi 2,07 mg Sn (tab. II, poz. 2).

Uzyskano odchylenie standardowe $S = 1,76$ i precyzję $2S = \pm 3,52\%$.

Oznacza to, że przy wykonaniu jednego oznaczenia największy błąd może wynosić $\pm 3,52\%$, zaś odpowiednio przy dwóch równoległych oznaczeniach $\pm 7,04\%$.

Zatem dokładność wyznaczania średniej z dwóch i więcej oznaczeń wynosi:

$$\text{dla 2 oznaczeń } \bar{y} = \frac{3,52}{\sqrt{2}} = \frac{3,52}{1,4142} = 2,49\%$$

$$\text{" 3 " } \bar{y} = \frac{3,52}{\sqrt{3}} = \frac{3,52}{1,7321} = 2,03\%$$

$$\text{" 4 " } \bar{y} = \frac{3,52}{\sqrt{4}} = \frac{3,52}{2} = 1,76\%$$

$$\text{" 5 " } \bar{y} = \frac{3,52}{\sqrt{5}} = \frac{3,52}{2,2361} = 1,57\%$$

A więc średnia z dwóch równoległych oznaczeń może być obarczona błędem $\pm 2,49\%$, zaś z 5 oznaczeń $\pm 1,57\%$.

Następnie wykonano 25 oznaczeń zawartości cyny w roztworze podstawowym Sn biorąc do każdego oznaczenia po 5,03 i dalej po 4,93 ml roztworu podstawowego co odpowiada 5,09 i 4,99 mg Sn (tab. II, poz. 3).

Uzyskano odchylenie standardowe $S = 1,14$ i precyzję $2S = 2,28\%$.

Oznacza to że przy wykonaniu jednego oznaczenia największy błąd może wynosić $\pm 2,28\%$, zaś odpowiednio przy dwóch równoległych oznaczeniach $\pm 4,56\%$.

A więc dokładność wyznaczania średniej z dwóch i więcej oznaczeń wynosi:

dla 2 oznaczeń $\bar{y} = \frac{2,23}{\sqrt{2}} = \frac{2,28}{1,4142} = 1,61\%$

" 3 " $\bar{y} = \frac{2,28}{\sqrt{3}} = \frac{2,28}{1,7321} = 1,32\%$

" 4 " $\bar{y} = \frac{2,28}{\sqrt{4}} = \frac{2,28}{2} = 1,14\%$

" 5 " $\bar{y} = \frac{2,28}{\sqrt{5}} = \frac{2,28}{2,2361} = 1,02\%$

Zatem średnia z dwóch równoległych oznaczeń może być obarczona błędem ±1,61%, zaś z 5 oznaczeń ±1,02%.

Następnie sprawdzono czy precyzja 2S = ±3,52 różni się istotnie od precyzji 2S = ±2,28.

Do sprawdzenia istotności różnic między precyzjami zastosowano test *F Snedecora* [7]:

$$F = \frac{S_{\bar{x}_1}^2}{S_{\bar{x}_2}^2},$$

w którym:

F — liczba *Snedecora*,

$S_{\bar{x}_1}^2$ — wariancja średniej arytmetycznej jednego szeregu,

$S_{\bar{x}_2}^2$ — " " " drugiego szeregu

Wariancję średniej arytmetycznej obliczano według następującego wzoru [7]:

$$S_{\bar{x}}^2 = \frac{S^2}{n}$$

w którym:

$S_{\bar{x}}^2$ — wariancja średniej arytmetycznej,

S^2 — " próby,

n — ilość oznaczeń.

Otrzymano wartość $F = 2,45 > F_{\alpha=0,05} = 1.96$ dla n = 24 i 25. Zaś $F = 2,45 < F_{\alpha=0,01} = 2.62$ dla n = 24 i 25.

W przeprowadzonym teście istotności mogą zajść trzy następujące przypadki:

1) wartość F obliczona na podstawie otrzymanych wyników oznaczeń jest mniejsza od wartości F odczytanej z tabel przy poziomie istotności 0,05. Oznaczenia są jednakowo precyzyjne;

2) wartość F z doświadczenia jest większa od wartości F z tabel przy poziomie istotności 0.05 a mniejsza od wartości F z tabel przy poziomie istotności 0.01. Oznaczenia można traktować jako w przybliżeniu jednakowo precyzyjne;

3) wartość F z doświadczenia jest większa od wartości F z tabel przy poziomie istotności 0.01. Oznaczenia wykonywane są z różną precyzją.

Tak więc w powyższym przypadku różnice między badanymi precyzjami są nieistotne, oznaczenia były wykonane w przybliżeniu z tą samą precyzją. A zatem można wyprowadzić wniosek, że precyzja zmodyfikowanej metody jodometrycznej przy zastosowaniu butli stalowej jako źródła CO_2 nie zależy od ilości cyny w zakresie stężeń od około 2 do około 5 mg Sn w próbce.

5 mg Sn w próbce.

WNIOSKI

1. Opracowana zmodyfikowana metoda oznaczania zawartości cyny pozwala na otrzymywanie poprawnych wyników przy zastosowaniu mnożnika poprawkowego. Dla metody tej otrzymano średni odzysk 95,86% oraz średnią wartość precyzji $2S = \pm 2{,}9\%$. Modyfikacja metody polega na zmniejszeniu pH środowiska reakcji oraz na zwiększeniu ilości środka redukującego sole cynowe do cynawych.

2. Stosując zmodyfikowaną metodę jodometryczną należy unikać używania aparatu *Kippa* jako źródła CO_2. Dobre wyniki daje zastosowanie w tym celu butli ze sprężonym dwutlenkiem węgla.

3. Opracowana modyfikacja metody jodometrycznej pozwala na uzyskanie poprawnych wyników jedynie w zakresie stężeń od 2 do 5 mg Sn w próbce.

4. Zmodyfikowana metoda jodometryczna oznaczania zawartości cyny jest obarczona niewielkim kierunkowym (ujemnym) błędem systematycznym. Obliczona średnia wartość mnożnika poprawkowego dla stężeń od 2 do 5 mg Sn w próbce wynosi 1,043.

5. Zmodyfikowaną metodę jodometryczną przy zastosowaniu mnożnika poprawkowego możnaby wprowadzić jako metodę obowiązującą do oznaczania zawartości cyny w artykułach żywności zamiast ciągle jeszcze stosowanej metody według PN-59/A-04014.

Б. Фитак, А. Райперт

ЙОДОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ СОДЕРЖАНИЯ ОЛОВА

II часть — Модификация йодометрического метода определения содержания олова.

Содержание

Разработан модифицированный йодометрический метод определения содержания олова (на чистых растворах). Модификация метода основывается на снижении pH реакционной среды путём добавления 50 мл соляной кислоты уд. вес 1,19 и 12 мл серной кислоты уд. вес 1,84 (вместо, как подает Польская Норма — 59/А-04014, 25 мл соляной кислоты и 6 мл серной кислоты) а также путём увеличения количества реагента (металлического алюминия), редуцирующего оловянные соли с превращением в оловянистые, с 0,4 до 0,6 г.

Модифицированный йодометрический метод определения содержания олова был проверён в условиях применения, в качестве источника двуокиси угля, аппарата Киппа и затем баллона со сжатым газом и концентрации олова в пробах в пределах с 0,5 по 10 мг. Проводился статистический анализ всех полученных результатов. Разработанный метод должен применяться в условиях использования стального баллона как источника CO_2 для определения олова в пределах содержания в пробе с 2 по 5 мг. В пределах этих концентраций воспроизводи-

мость в среднем достигает 95,86%, средняя точность достигает ±2.9% и средний корригирующий коэффицент „к" равняется 1,043.

Предлагается применить представленный метод в практике осуществления лабораторного контроля качества пищевых продуктов вместо обязывающего йодометрического метода, указанного в ПН-59/А-04014.

B. Fitak, A. Rajpert

A IODOMETRIC METHOD FOR THE ASSAY OF TIN CONTENTS

II. A Modification to the Iodometric Method for the Assay of the Contents of Tin

Summary

A modified iodometric method of tin determination was elaborated on pure solutions. The modification introduced to the method consists in lowering the pH of the reaction medium by addition of 50 ml HCl density 1.19 and 12 ml H_2SO_4 density 1.84 in place of 25 ml of HCl and 6 ml of H_2SO_4 according to the Polish Standard PN-59/A-04014. Furthermore, the amount of the reducing agent (metallic aluminium) used for the reduction of stannic salts to stannous salts was increased from 0.4 g to 0.6 g.

The modified iodometric method for the assay of tin was tested at concentrations ranging from 0.5 mg to 10 mg of tin per sample using as the CO_2 sources a Kipp generator and a cylinder with compressed CO_2. The average recovery at the concentrations mentioned amounted to 95.86%, the mean value of precision 2S being ±2.9% while the mean coefficient of correction was k = 1.043.

The method is recommended for the use in food inspection laboratories in the place of the iodometric method introduced by the Polish Standard PN-59/A-04014.

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Methods of Evaluating Tin and Fluoride Salts as Anticaries Agents in Animal Caries Experiments

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The susceptibility of rat molar teeth to the development of carious lesions under proper experimental conditions of diet[1] and presence of oral flora[2] is an accepted fact. It has been shown that fracture by mastication[3] is not necessary for the production of carious lesions in the enamel.[4,5] Anticaries agents can be administered in the food or water or can be applied topically with a swab or brush. To test systemic anticaries effects, agents have been injected intraperitoneally or subcutaneously. Application of materials by the method of stomach tubing is a technique that has also been used. All these methods have certain advantages and certain drawbacks and care must be used in interpreting the results.[5]

Comprehensive reviews have been written on the early work concerning the relation of fluorine compounds to dental caries experience in man[7] and animals.[6,7] The effect of administering a wide spectrum of caries inhibiting agents by a variety of methods has recently been reviewed by Shaw.[8,9]

The objectives of this paper are as follows: (1) to determine the anticaries effectiveness of stannous chloride, sodium fluoride, and stannous fluoride; (2) to compare the above compounds by the administration in the drinking water and by application topically; (3) to determine the effect of frequency of application of anticaries agents; and (4) to relate the caries incidence in the first, second, and third molars to systemic versus topical effects of tin and fluoride salts.

Materials and Methods

ANIMAL REGIMEN.—Weanling Wistar strain rats* (23–25 days old, 60 ± 10 gm. weight) were housed individually on raised stainless steel wire bottom cages. The animals were placed immediately on a modified Stephan diet #580,[10] consisting of nonfat dry milk 32, sucrose 63, dried liver extract 2, and Celluflour 3 per cent, respectively. Water and food were administered ad libitum. The experiments were allowed to continue until the state of caries development in the control group reached the extent desired, as determined by in vivo examination under the dissecting microscope. All animals were killed with chloroform and were autoclaved to obtain the clean femora and jawbones.

Three separate experiments are here reported. Experiment I had a duration of 119 days. The anticaries agents were fed at low concentration in the drinking water ad libitum. Experiment II had a duration of 77 days. The anticaries agents were applied at high concentration topically once per week with a total of 10 topicals. Equal numbers of male and female animals were assigned by random selection to each treatment group with 30 animals per group in both experiments. Experiment III had a duration of 85 days and was designed so that three frequencies of application, three compounds, three concentrations, and two methods of application were investigated. The design was a duplicated $3 \times 3 \times 3 \times 2$ factorial experiment confounded in litters of six. In both replicates six litter mates per block were assigned in accordance with the first half of the Z design for a $3 \times 3 \times 3 \times 2$ factorial as described by Yates.[11] Male and female rats were assigned to treatment combinations at random. The factors are shown in Table 1. Topical treatments were carried out over a period of about 8 weeks. Animals which were treated only once were treated on the first day of topicals.

ADMINISTRATION OF ANTICARIES AGENTS

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TABLE 1

DESIGN OF FACTORS IN EXPERIMENT III: EXPERIMENTAL FACTORS

Compound	Frequencies of Application	Concentrations (Per Cent)	Methods
SnF_2	Once only, once/week, daily	7.43, 0.37, 0.093	Wet, dry
NaF	Once only, once/week, daily	4, 0.20, 0.05	Wet, dry
H_2O	Once only, once/week, daily	.....	Wet, dry

IN THE DRINKING WATER.—The agents were dissolved in the drinking water of the animals and were supplied ad libitum. In the case of the tin salts, rigid precautions were taken to attempt to protect the very low concentrations of Sn(II) against oxidation and hydrolysis before the animal had a chance to drink the solution. Determination of the stannous ion concentrations were made on the stock solutions, on the freshly dispensed solutions (10 minutes after dispensing to the water bottle and placing on the rack), and on the solutions at various times in the 24 hour period before refilling with fresh solutions. Sufficient solution for several days consumption was prepared at one time using oxygen-free nitrogen[12] to scavenge the water and protect the solutions until they were dispensed to the individual water bottles. Water bottles were emptied and refilled with fresh solution once a day, 7 days a week. Daily records of the water consumption were kept for a period of 17 weeks to insure that the average volume of consumption in the four groups was equal. The stannous chloride and stannous fluoride solutions were made up at 31 ppm Sn(II) and the sodium fluoride and stannous fluoride at 10 ppm F.

ADMINISTRATION OF ANTICARIES AGENTS BY TOPICAL APPLICATION.—The material being tested was topically applied to the molar teeth of rats with a small cotton swab while the animal was immobilized and jaws retracted by a device similar to the one devised by Johansen.[13] The advantage of topical application is that all quadrants of the jaw can be more uniformly applied than by water feeding and very high or very low concentrations of agents can be applied as fresh solutions at known concentrations. Furthermore, the actual number of applications and time of application then are under control of the investigator.

CARIES EVALUATION.—The clean rat jaws were examined carefully with a binocular microscope* at thirty power and gross carious lesions were graded using the 1+ to 5+ severity scores of Shaw.[14] In teeth which were perfect or only slightly damaged, each cusp was fractured off by a small wedge so that the base of each fissure could be examined for minor carious lesions.

ANALYTICAL METHODS.—Concentrations of Sn(II) in the aqueous solutions were determined by the standard iodimetric method using a starch indicator.[15] The femora were dried and ground and the total tin content was determined by emission spectroscopy.[16] Fluoride,[17] calcium,[18] and phosphorus[19] contents of the femora were also determined.

Results

THE ANTICARIOGENIC EFFECTIVENESS OF SnF_2, $SnCl_2$, and NaF AT LOW CONCENTRATION IN THE DRINKING WATER (EXPERIMENT I).—Since stannous fluoride and stannous chloride solutions are known to be subject to both hydrolysis and oxidation, analyses were made in Experiment I for Sn(II) concentrations on the original stock solutions; the solutions after dispensing into the water bottle, stoppering, and inverting on the rack; and at various times through the 24 hour period before the next day's water change. The results of these analyses are shown in Figure 1. The dispensing alone caused about a 25 per cent loss in concentration of Sn(II) in the two tin solutions. In the subsequent 24 hour period it is evident that the stannous chloride was extremely unstable, having no titratable Sn(II) after about eight hours on the rack. With stannous fluoride, on the other hand, although there was essentially a constant rate of loss, 23 per cent of the original Sn(II) still remained at 24 hours. A slight amount of fluoride from stannous fluoride was lost by

* Cyclopic model, American Optical Co., Buffalo, N.Y.

occlusion in the hydrated stannous oxide which precipitates from the hydrolyzing solution. Sodium fluoride under the above conditions was stable. Because of these oxidation and hydrolysis effects with the tin salts, a serious problem not generally recognized, it is not possible to say that the stannous chloride, stannous fluoride, and sodium fluoride were administered to the animals at equivalent Sn(II) and fluoride concentrations.

The caries scores from this experiment based on gross carious lesions are shown in Table 2. While stannous fluoride effected a significant reduction in caries score, neither stannous chloride nor sodium fluoride had an effect. Analysis of the carious molars affected, number of carious lesions involved, and the caries scores gave essentially the same result. There was no significant difference in water consumption, food consumption, or body weight gain in the four experimental treatment groups. Thus, based on water consumption there is a valid comparison among the treatment groups as determined by total cumulative consumption of the agents in the water. The effective concentrations of the tin solutions, however, were quite different, as indicated in Figure 1.

TABLE 2

THE EFFECTIVENESS OF Sn(II) AND F^- AT LOW CONCENTRATION IN DRINKING WATER IN EXPERIMENT I

(Original Strength: 31 ppm Sn(II), 10 ppm F^-)

Water Solution	Caries Score*	Per Cent Reduction in Score
SnF_2	43	31
NaF	57	9
$SnCl_2$	61	2
H_2O	62	.....

* Least significant difference (l.s.d.) required is 15 caries score units

THE ANTICARIES EFFECTIVENESS OF TOPICAL APPLICATIONS OF 2 PER CENT SnF_2, 2 PER CENT $SnCl_2$, AND 2 PER CENT NaF (EXPERIMENT II).—Since the administration of known low concentrations of tin compounds in the drinking water was not possible (Fig. 1), topical applications of agents at known concentrations seemed the most logical approach to the problem. In Experiment II, 2 per cent solutions of stannous fluoride,

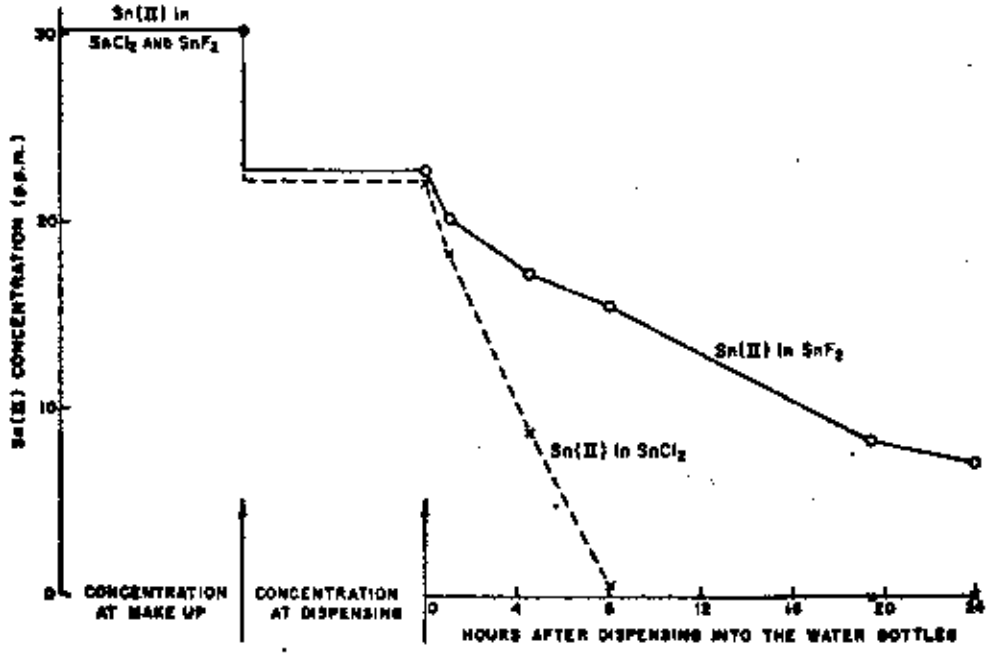


FIG. 1.—Concentration of Sn(II) available to animals from water solutions over a 24-hour period

stannous chloride, or sodium fluoride were applied once per week to rats from weaning age. Water was applied in exactly the same manner to the fourth group. A summary of the data is presented in Table 3. Stannous fluoride and sodium fluoride exerted a significant effect in reducing the incidence of gross carious lesions in the rats when compared to the stannous chloride and water. There was no statistical difference between the effectiveness of stannous fluoride and sodium fluoride. The reduction in caries produced by stannous chloride was not statistically significant. The sex of the animals did not affect caries score nor were there any interactions among the experimental variables. There was no change in significance of any of the comparisons

TABLE 3

THE TOPICAL EFFECTIVENESS OF 2 PER CENT SnF_2, 2 PER CENT $SnCl_2$, AND 2 PER CENT NaF IN EXPERIMENT II

Material Applied	Caries Score*	Per Cent Reduction in Score
NaF (10,000 ppm F^-)	19	50
SnF_2 (5,000 ppm F^-)	24	50
$SnCl_2$	40	16
H_2O	48	.....

* Least significant difference (L.s.d.) required is 14 caries score units.

whether made by caries score, lesions, or molars. Cumulative body weight gains were not significantly different among the four topical groups.

THE INFLUENCE OF TOPICAL APPLICATION FREQUENCY, METHOD, AND CONCENTRATION OF TIN AND FLUORIDE SALTS (EXPERIMENT III).—In in vitro systems it was determined that the frequency of application of a material such as stannous fluoride at low concentration made a significant difference in the protection afforded against decalcification, decalcification decreasing with increasing frequency of treatment.[20] Human clinical topical applications of NaF[21–23] and tests of controlled frequency of brushing with dentifrices containing SnF_2[24] have also indicated that frequency of application may be an important influencing factor. Topicals in humans are followed by drying the material on the teeth after application.[25] Whether this increases or decreases the effectiveness of the treatment has not been reported but could make a difference with a material such as stannous fluoride which is easily oxidized. Previous in vitro studies, however, have indicated that once teeth have been treated with stannous fluoride or stannous chloride, the tin in the tooth structure was very stable and resisted oxidation for long periods.[20] This third animal experiment was designed to investigate the influence of some of the variables involved in applying therapeutic materials to the teeth of rats.

In Experiment III the caries score averages for the effect of compounds and for the effect of frequency of application are shown in the bar chart in Figure 2. The shaded area at the top of each bar indicates the 95 per cent confidence limits on the means of the caries scores. Per cent reduction in caries score listed by compounds (across all other factors), based on the water control, and the per cent reduction produced by increasing frequency of topicals (across all other factors), based on a single topical, are given in Table 4. Both stannous fluoride and sodium fluoride treatments produced significant reductions in caries even though the reductions are the averages across three concentrations and three frequencies. The marked effect of frequency (49 per cent reduction on daily treatment) when compared to a single treatment is even more dramatic when it is realized that this was effected by only two out of the three treatment compounds since the third compound was distilled water.*

The differences in effectiveness of the three concentration levels of stannous fluoride and sodium fluoride are shown in Table 5. The difference in effectiveness of the above two materials is essentially the same as that observed when they were tested in the agar-saliva in vitro system,[20] where stannous fluoride and sodium fluoride were not markedly different at the 2 per cent level, but 0.4 per cent stannous fluoride was decidedly more protective in vitro than was 0.2 per cent sodium fluoride.

* While this animal caries experiment was useful, it was not particularly efficient since the experiment was heavily weighted with treatment combinations which were ineffective. The main reason for the "dilution" effect was, of course, the use of a control, water, as one treatment group, the problems of which are discussed in O. Kempthorne's *The Design and Analysis of Experiments* (New York: John Wiley & Sons, Inc., 1952), pp. 304–09.

TABLE 4

THE EFFECT OF COMPOUNDS AND FREQUENCY ON CARIES—EXPERIMENT III

Compounds	Frequencies	Per Cent Reduction
SnF_2	Once only, once/week, daily	23
NaF	Once only, once/week, daily	17
H_2O	Once only, once/week, daily	.....
SnF_2, NaF, H_2O	Daily	40
SnF_2, NaF, H_2O	Once/week	13
SnF_2, NaF, H_2O	Once only	.....

TABLE 5

THE EFFECT OF THREE EQUIVALENT FLUORIDE CONCENTRATION LEVELS OF SnF_2 AND NaF ON REDUCTION OF CARIES SCORES—EXPERIMENT III

SnF_2		NaF	
Per Cent Concentration	Per Cent Reduction of Caries Score	Per Cent Concentration	Per Cent Reduction of Caries Score
7.46 (~20,000 ppm F^-)	40	4.0 (~20,000 ppm F^-)	29
0.37 (~1,000 ppm F^-)	28	0.2 (~1,000 ppm F^-)	16
0.093 (~250 ppm F^-)	19	0.05 (~250 ppm F^-)	7

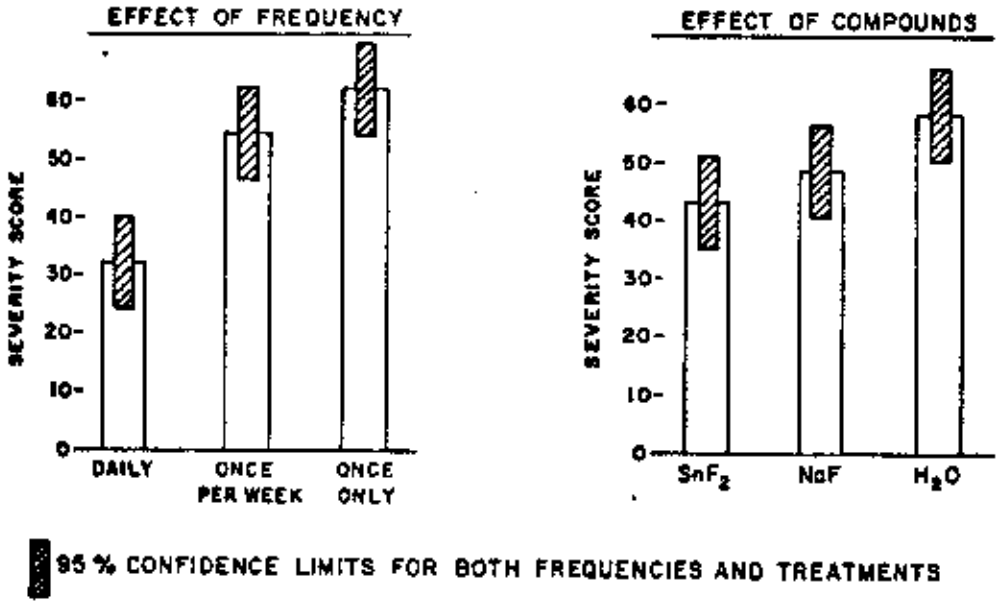


FIG. 2.—The effect of frequency of application and of compound applied on the caries score. The shaded area represents the 95 per cent confidence limits on the means of the caries scores.

In this experiment it was found also that the caries score varied significantly with litters. There was no significant effect of drying the teeth with a blast of air after application of the agents.

Body weight gain was significantly lower in the stannous fluoride and high frequency (once per day) group. There was a lower food consumption in the latter group also. There was no significant difference, however, in body weight gain or food consumption among animals in the other two frequency groups (once only and once per week) or compound groups (sodium fluoride and water), although caries incidence was quite different (Table 4) between each of these two comparisons. This suggests that differences in caries among the various groups are probably not due to food consumption but rather to the topical application of sodium and stannous fluorides at the different frequencies.

Discussion

In the evaluation of anticaries agents, different considerations were brought out by the three experiments here reported. The effect of frequency of application was particularly striking. Concentration of application, in combination with frequency, is particularly important in differentiating the efficacy of the two materials, stannous fluoride and sodium fluoride. At the very low level in the drinking water (10 ppm F^-), stannous fluoride still showed a strong caries inhibiting effect (31 per cent reduction), while sodium fluoride did not exert a significant effect (9 per cent reduction). These results agree in general with those published by Muhler, Nebergall, and Day,[20, 27] who administered similar levels of NaF and SnF_2 to rats on a coarse corn diet. The reason for the high level of reductions at the low concentration of stannous fluoride used in the water feeding experiment is probably related to frequency. In Experiment III the per cent reductions are across all three frequencies and thus one-third of the group received only one topical, while in Experiment I, the animals probably drank the water in the bottles as often as six to fifteen times a day.[28] Considering the profound effect of frequency shown in Experiment III, it is not too surprising that the more effective stannous fluoride showed a real reduction in caries in spite of the hydrolysis and oxidation of Sn(II) shown in Figure 1.

The difference in calcification and eruption times of the three rat molars may make a difference in the reaction of the teeth to treatment and hence in caries susceptibility of one molar versus another.[8] Using the data obtained in the water feeding and topical experiments, the caries scores of the first, second, and third molars are shown in the bar graphs (Figs. 3 and 4) as a function of treatment. The per cent reduction in caries based on the water control for each molar is shown in Table 6 for both Experiments I and II. From the table it is immediately evi-

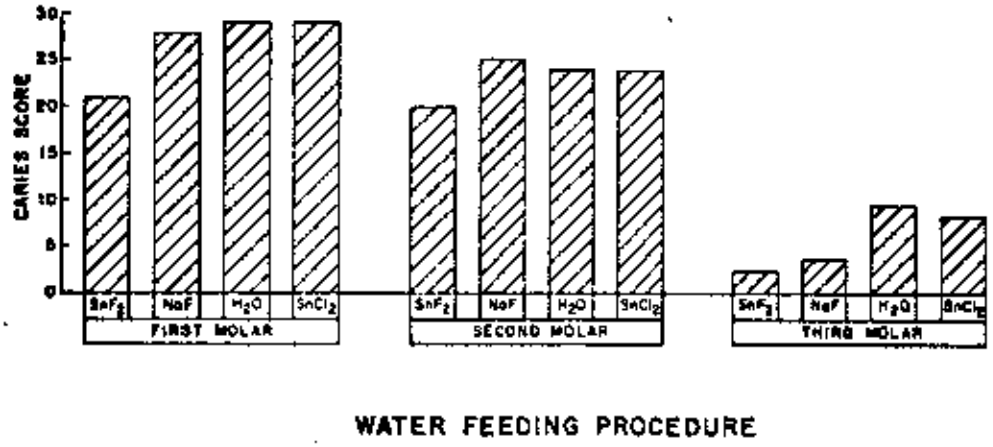


FIG. 3.—Caries scores for first, second, and third molars of rats receiving 10 ppm F and/or 31 ppm Sn(II) in their drinking water.

TABLE 6

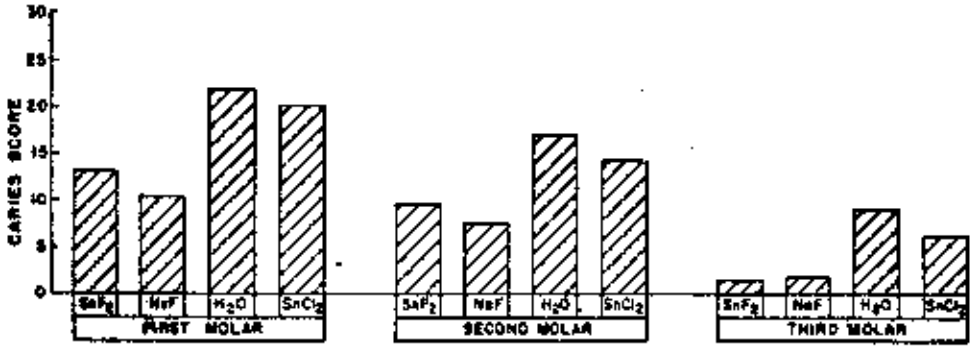
THE EFFECTIVENESS OF ANTICARIES AGENTS FOR EACH MOLAR
(Per Cent Reduction in Caries)

Agent	Experiment I — Water Feeding Method			Experiment II — Topical Method		
	First Molar	Second Molar	Third Molar	First Molar	Second Molar	Third Molar
NaF	3	5	59	52	57	81
SnF_2	28	16	77	41	44	84
$SnCl_2$	0	0	12	8	17	32
H_2O	.....	.....	.....	.....	.....	.....

dent that treatments by stannous fluoride, sodium fluoride, and even stannous chloride were most effective on the third molar. A comparison by Students' *t*-test of the third molar caries scores for animals on topical stannous chloride versus animals on water shows that the 32 per cent reduction was significant at better than the 98 per cent confidence level. The 59 per cent reduction of caries in the third molars of animals on the sodium fluoride solution in the water feeding method was also highly significant. In all the tin and fluoride treated groups there was a greater response in the third molar than there was in the first and second. This agrees with known clinical results in that children's teeth receiving topical treatments from eruption, or teeth of children receiving fluoridated water from birth, are more receptive to the inhibiting effects of fluoride than mature teeth.[29] The third molars of the rats in these experiments erupted about 10 days after the start of the topicals and the cariogenic diet, as opposed to the first and second molars which were erupted several days before the regimen started.

It is known that the lower molars of rats tend to decay more extensively than the upper molars.[11] In both Experiments I and II the caries incidence in the mandibular quadrants was higher than the caries incidence in the maxillary quadrants, which agrees well with the reported literature.

In the water feeding experiment it was known that tin and fluoride were being ingested, and it was suspected that in the topicals the animals were probably swallowing some of the strong solutions. Since the third molar was not erupted at the start of



TOPICAL APPLICATION PROCEDURE

FIG. 4.—Caries scores for first, second, and third molars of rats treated with 2 per cent SnF_2, 2 per cent NaF, 2 per cent $SnCl_2$, or with water.

TABLE 7

THE RELATION OF SKELETAL Ca, P, Sn, AND F TO TREATMENTS

Agent	Experiment I—Water Feeding Method					Experiment II—Topical Method				
	Ca (Per Cent) (1)	P (Per Cent) (2)	Ca/P (3)	Sn (ppm) (4)	F (ppm) (5)	Ca (Per Cent) (6)	P (Per Cent) (7)	Ca/P (8)	Sn (ppm) (9)	F (ppm) (10)
SnF_2	24.1	11.6	2.1	3.8	589	22.2	10.6	2.1	12.2	265
NaF	24.2	11.5	2.1	2.2	602	23.0	11.3	2.0	2.9	762
$SnCl_2$	24.0	11.6	2.1	2.2	14	22.3	11.1	2.0	0.7	21
H_2O	24.2	11.7	2.1	1.6	16	23.0	11.3	2.0	1.9	17

the experiments, the possibility of a systemic effect on the third molar was considered. Analyses were performed on the ground femora from Experiments I and II for calcium, phosphorus, tin, and fluoride and are shown in Table 7. In both experiments the Ca/P weight ratio was constant at 2.0–2.1 (cols. 3 and 8). This is a normal Ca/P ratio, and the calcium and phosphorus levels were very close to those found for human bone (Ca = 23.8 per cent, P = 10.4 per cent).[40] It is interesting to note that a small amount of tin did accumulate in the femora when small amounts, but high concentrations were ingested (col. 9), whereas, at the low level in the drinking water (.31 ppm Sn(II)), with daily intake, there was only a very small and questionably significant rise in tin concentration in the femora (col. 4). In contrast to this, the femora of the water feeding experiment accumulated almost as much fluoride as the topical experiment (col. 5 v. col. 10), showing the efficient fluoride storing capacity of the skeleton. The most probable reason for the difference between sodium fluoride and stannous fluoride storage in the topical experiment (col. 10) was the fact that both stannous fluoride and sodium fluoride were applied at the 2 per cent level and, therefore, about twice the fluoride concentration was applied in the sodium fluoride topical. The fact that in most experiments large amounts of fluoride were found in the skeleton suggests that for the third molar, at least, the systemic effect of fluoride cannot be ruled out as a possible factor in reducing caries, particularly in view of the differences in caries reduction observed in the third versus the first and second molars (Table 6). However, a re-evaluation of the caries reducing effect of stannous fluoride and sodium fluoride in Experiments I and II showed that the conclusions drawn on the basis of all three molars held true also for only the first and second molars, where it was presumed that the caries reduction was due solely to a topical effect.

Summary

The anticaries effectiveness of stannous chloride, stannous fluoride, and of sodium fluoride was investigated by administering the materials at low concentration in the water or by topically applying these materials at several concentrations and frequencies. If a material is unstable, such as stannous chloride or stannous fluoride, topical application was the method of choice so that concentrations of application at realistic levels could be effectively controlled. With topical applications at equivalent low concentrations of fluoride (0.1 per cent SnF_2, 0.05 per cent NaF), SnF_2 was found to be more effective than NaF. At high concentrations, however (2 per cent and 8 per cent SnF_2, 2 per cent and 4 per cent NaF), these two materials were about equal in their caries reducing effect in rats. Stannous chloride applied topically at 2 per cent was ineffective. Stannous fluoride supplied at 10 ppm fluoride in drinking water was markedly superior to sodium fluoride at at the same fluoride concentration while stannous chloride at a similar concentration of Sn(II) to stannous fluoride was ineffective.

The frequency of application of topically effective agents was an extremely important variable in determining caries progression.

The more frequent the application, the more effective the caries reduction was found to be.

There was an individuality of response of the first, second, and third molar to the application of anticaries agents. Stannous chloride, stannous fluoride, and sodium fluoride were most effective on the third molar.

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DETERMINING TIN IN FOOD PRODUCTS BY THE DITHIOL METHOD USING "MERSAPON" DETERGENT

by

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Laboratory Notes

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Dr. R. Hejmo, Director

The method of using dithiol in determining tin in foodstuffs was described in detail by Bernstein and Gilewska in the PZH yearbook #3 (1954) and #3 (1955). The authors recommend the use of "Mersolan" as an appropriate dispersing agent to prevent coagulation of sediment of the red group of stannous mercaptide.

It was found that the Bydgoski Fat Industry Company had stopped producing "Mersolan". However, since a dispersin agent is required in determining tin by the dithil method in a photoelectric colorimeter, it has now been found useful to replace "Mersolan" with another dispersin agent.

From a number of natively-manufactured detergents tested (FF, Szampon, Mersapon) "Mersapon" was selected;

it is produced by the Polish Chemical-Pharmaceutical Labor Cooperative in Lodz. Through precise verification it was found that "Mersapon" (an aqueous solution of a mixutre of alkylsulfonates with carbon bonds C_{12}-C_{18}) is an equivalent of "Mersolan", i.e., it contains the same superficial tin agent.

Since "Mersapon" is a detergent produced by a different plant, a meeting was held to verify its suitability for the colorimetric method of determining tin.

A series of tests were run on known concentrations of tin and reaction with tin was effected after Bernstein and Gilewska, the only difference being that instead of "Mersolan" solution, a solution of "Mersapon" was used, in differing concentrations and quantities: 1, 0.7, 0.5, 0.3, or 0.2ml.

It was found that freshly-diluted "Mersapon", added to test solutions in amounts of 0.5, 0.3, or 0.2ml (2ml of commercial "Mersapon" supplemented with water to make 100ml) displays the best dispering characteristics.

Solutions remain clear for 8-12 hours. Wider difference bands on the color scale were established which are important in visual comparison for tin content.

A determination using a photoelectric colorimeter (Model 581, made in China) was made, profiling the specimen curve. Ten series of specimen solutions were prepared, each containing 0; 5; 10; 15;...100μg of tin, with an additional 0.5ml of "Mersapon" solution (2/100); 5 series of tests with addition of 0.3ml Mersapon; and 5 series with addition of 0.2ml of Mersapon solution.

In all cases, the graph of extinction as a function of solution concentration comprised a straight line (Fig. 1)

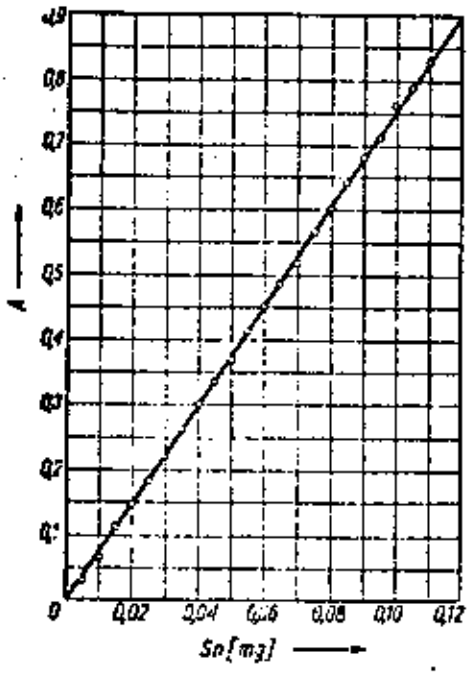

Fig. 1. Specimen Curve of Tins with Mersapon (0.5ml Mersapon 2/100 in 7ml solution). Determination made on Model 581 Photocolorimeter.

At the MSSE Food Research Laboratory in Gdynia, determination of tin content by the dithiol method using a "Mersapon" additive has been conducted since January, 1965.

On the basis of many tin content determinations - in preserved fish, meat, vegetable, and fruits of national and foreign origin - the high suitability of the detergent under discussion has been established.

CONCLUSIONS

"Mersapon" satisfies conditions for a good dispersing agent for the dithiol method of determining tin:

1. it retains stannous mercaptide sediment in a state of dispersion for more than ten hours;
2. transparent solutions of stannous dithiolan with "Mersapon" yield to the Lambert-Beer law.

And consequently, a "Mersapon" solution can be used for determining tin visually as well as by photocolorimetry.

The introduction into Polish Standards of dithiol methods now in popular use is assumed - with the readily available "Mersapon" of native manufacture, for determining tin in foodstuffs.

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OLIMPIA GAJEK, HELENA KLIMCZAK

OZNACZANIE CYNY W ŻYWNOŚCI METODĄ DITIOLOWĄ PRZY UŻYCIU DETERGENTA O NAZWIE „MERSAPON"

NOTATKA LABORATORYJNA

Z Pracowni Badania Żywności MSSE w Gdyni
Dyrektor: dr *R. Hejmo*

Metoda oznaczania cyny w żywności za pomocą ditiolu została dokładnie opisana przez *Bernstein* i *Gilewską* w Rocznikach PZH nr 3, 1954 i nr 3, 1955 r. Autorki polecają stosowanie „Mersolanu" jako odpowiedniego środka rozpraszającego w celu zapobieżenia koagulacji osadu czerwonego kompleksu merkaptydu cyny.

Okazało się, że Bydgoskie Zakłady Przemysłu Tłuszczowego zaprzestały produkcji „Mersolanu". Ponieważ jednak przy oznaczaniu cyny metodą ditiolową na kolorymetrze fotoelektrycznym środek rozpraszający jest niezbędny, postanowiono używany dotychczas „Mersolan" zastąpić innym środkiem rozpraszającym.

Spośród przebadanych detergentów produkcji krajowej (FF, Szampon, Mersapon) wybrano „Mersapon" produkowany przez Spółdzielnię Pracy Chemiczno-Farmaceutyczną Polon w Łodzi. Po dokładnym sprawdzeniu okazało się, że „Mersapon" (roztwór wodny mieszaniny alkilosulfonianów o łańcuchach węglowych C_{12} — C_{18}) jest odpowiednikiem „Mersolanu" tzn. zawiera ten sam środek powierzchniowo czynny.

Ponieważ „Mersapon" jest detergentem produkowanym przez inną wytwórnię postanowiono więc sprawdzić jego przydatność w kolorymetrycznej metodzie oznaczania cyny.

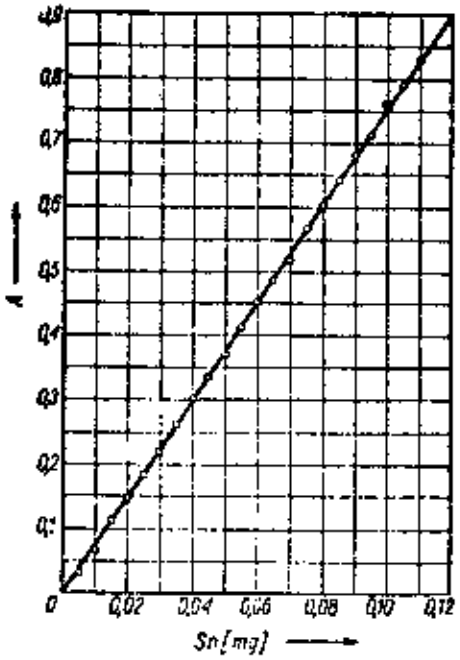
Przygotowano szereg prób o znanych stężeniach cyny i przeprowadzono reakcję z ditiolem wg *Bernstein* i *Gilewskiej* z tą tylko różnicą, że zamiast roztworu „Mersolanu" dodawano roztwór „Mersaponu" w różnych stężeniach i ilościach: 1, 0,7, 0,5, 0,3 lub 0,2 ml.

Okazało się, że najlepsze właściwości rozpraszające wykazuje „Mersapon" świeżo rozcieńczony (2 ml „Mersaponu" handlowego uzupełnione wodą do objętości 100 ml) i dodany do roztworów badanych w ilości 0,5, 0,3 lub 0,2 ml.

Roztwory pozostają klarowne od 8—12 godz. Stwierdzono też większe rozpiętości różnic barwnych skali, co jest ważne przy porównywaniu wizualnym zawartości cyny.

Przeprowadzono także oznaczenia w kolorymetrze fotoelektrycznym (model 581 produkcji chińskiej), wykreślając krzywe wzorcowe. Przygotowano 10 serii roztworów wzorcowych, z których każda zawierała 0; 5; 10; 15; — 100 µg cyny z dodatkiem 0,5 ml roztworu „Mersaponu" (2/100); 5 serii z dodatkiem 0,3 ml roztworu „Mersaponu" i 5 z dodatkiem 0,2 ml roztworu „Mersaponu".

We wszystkich przypadkach wykres zależności ekstynkcji od stężenia roztworów wzorcowych stanowi linię prostą. Ryc. 1.



Ryc. 1. Krzywa wzorcowa cyny z „Mersaponem" (0,5 ml „Mersaponu" 2/100 w 7 ml roztworu). Oznaczenia przeprowadzono na fotokolorymetrze model 581.

W Laboratorium Badania Żywności MSSE w Gdyni prowadzi się oznaczenia zawartości cyny metodą ditiolową z dodatkiem „Mersaponu" już od stycznia 1965 r.

Stwierdzono na podstawie wielu oznaczeń zawartości cyny — w konserwach rybnych, mięsnych, warzywnych i owocowych produkcji krajowej i zagranicznej — dużą przydatność omawianego detergentu.

WNIOSKI

„Mersapon" spełnia warunki dobrego środka rozpraszającego w ditiolowej metodzie oznaczania cyny:

1) utrzymuje osad merkaptydu cyny w stanie rozproszenia przez kilkanaście godzin;

2) klarowne roztwory ditiolanu cynawego z „Mersaponem" podlegają prawu Lamberta-Beera.

A więc roztwór „Mersaponu" może być użyty zarówno do oznaczania cyny wizualnie, jak też i fotokolorymetrycznie.

Proponuje się wprowadzenie do Polskiej Normy szeroko stosowanej obecnie metody ditiolowej — z łatwo dostępnym „Mersaponem" produkcji krajowej — do oznaczania cyny w żywności.

О. Гаек, Х. Климчак

ОПРЕДЕЛЕНИЕ ОЛОВА В ПИЩЕВЫХ ПРОДУКТАХ ДИТИОЛОВЫМ МЕТОДОМ ПРИМЕНЯЯ ДЕТЕРГЕНТ НАЗЫВАМЫЙ „MERSAPON"

O. Gajek, H. Klimczak

THE DETERMINATION OF TIN BY THE DITHIOL EMPLOYING APPLICATION OF THE DETERGENT MERSAPON

Dn. 9.XII.1965 r.

Gdynia, ul. Chrzanowskiego 19.

A DRY MINERALIZATION METHOD USED IN DETERMINING TIN IN FOODSTUFFS

by

Olimpia Gajek & Helena Klimczak

From a research report of the Sanitary-Epidemiology Port Station in Gdynia, (Director Dr. J. Stankiewicz).

In studying foodstuffs for a content of heavy metals which are injurious to health, there most often arises a need to assay tin content.

For example, in food products stored in stamped preserve jars which are tinned and not lacquered, or have a lacquer defect, there is no particular need to assay lead, copper, or zinc, but mainly tin. In foodstuffs stored in a collapsible preserve can where there is no solder-seal leakage to within or where a quality test confirms an undesirable trace of it in the upper or lower part of the container seam, analysis is also conducted for tin since, as experience shows, in production there is no lead at all or at the most, only a trace.

Until now, the rules governing us (Polish Standards, Foodstuffs Analysis Manuals) for the assay of heavy metals in foodstuffs - except for zinc - have

demanded the wet mineralization method.

It is well-known that the wet mineralization method requires a relatively long length of time (especially for products such as fish or meat), and requires a significant quantity of reagent, etc. as well as - what is most important from the viewpoint of the nitric oxide liberated which is injurious to health - good extraction or a special mixture, which does not always lie within the technical resources of every smaller food inspection laboratory.

Because tin content is most often assayed in survey inspections of foodstuffs - as has been discussed above - it was undertaken to test the replacement of the wet mineralization by a dry mineralization method which reveals the smallest excess of tin.

For this purpose, a series of different food products was incinerated; certain quantities of a standard tin solution were added to this and the tin content was assayed in the dissolved ash by the dithiol method [1] using the detergent "Mersapon" [2]. The ash was dissolved in various ways, as discussed in the experimental portion of this paper.

A standard tin solution scale was prepared for each assay series which was compared with test samples visually and by using a Chinese Model 581 photocolorimeter which uses a green filter; or the Zeiss Company's SPEKOL with a wave length of 535mμ [3].

Experimental Part

1. To tomato paste which has been verified in advance, we add a certain quantity of standard tin solution and, after precise pulverization in a mortar, we weigh out a sample of 10 and 20 grams in a porcelain steam dish; this is then inserted into a dessicator at 100°C (for about 4 hours) and is then further dried on an electric plate (on an asbestos mesh); there it is carefully carbonized by a small flame until smoldering and is then incinerated in a muffle furnace at 450-500°C.

The resulting ash (grey color) is carefully transferred to an iron crucible, at the bottom of which are placed 5-8 grains (depending on the quantity of ash) of sodium hydroxide [4]; this is melted over a flame (transforming tin to a stannous suspension in water) [5].

Upon cooling, a certain amount (about 5 ml) of distilled water is added to the crucible; this is carefully heated and then the contents of the crucible are transferred (quantitatively) to an ash-free filter; it is filtered into a 100 ml measuring flask, washing the crucible and filter with hot water several times to fill the flask to about 2/3 capacity.

Then two drops of phenophthalein solution are added to the flask containing the filtered solution; this is acidified by HCl to total decolorization (tin is transformed into stannic chloride). After cooling the flask contents to 20°C, distilled water is added to make 100 ml. A pipette of 1 or 2ml of the solution prepared in this manner (depending on the amount of tin added) is taken and tin content is assayed by the dithiol method [1].

In the same way, an experiment is conducted with samples of banana compote and herring in oil. By visual comparison of these tests with standard scales, very small differences (not yielding to precise determination) in the intensity of blue tin mercaptide were noticed in a few test tubes. These differences, accurately interpreted using a photocolorimeter, are shown in Table 1.

Table 1

Tin Assay Findings in Products Which Have Been Dry Mineralized and Melted with Hydroxide

Product	Quantity of Preserved Food Used in Incineration, gr.	Sn Added to Sample, mg	Quantity Sn with Control Test in Conversion for 1kg, mg	Sn per 1 kg, mg	% Error
tomato paste	20	---	---	0	---
"	20	4	200	200.0	0.0
"	10	2	200	185.0	-7.5
"	10	2	200	195.0	-2.5
Banana compote	20	---	---	42.5	---
"	20	1	92.5	85.0	-8.1
"	20	1	92.5	87.5	-5.4
"	20	1	92.5	88.7	-3.8
Herring in oil	20	---	---	0	---
"	20	4	200	185.0	-7.5
"	20	4	200	205.0	+2.5
"	10	2	200	181.6	-9.2

As can be seen from the table, the results are fairly good - error is less than 10%. But in order to achieve these results, we had to conduct a series of tests to identify and improve the weak points in the method: this requires quite a bit of work, such as: transfer of ash from steam chamber to crucible, melting with hydroxide, quantitative transfer of crucible contents to flask, and acidification to proper pH to avoid iridescence or sedimentation (the addition of phenophthalein aided us very much in this respect).

Moreover, if the ash contains traces of carbonized product (unincinerated), then during melting with the hydroxide a brown color appears while passing through the filter and this test is no longer reliable for colorimetric assays. There is thus a search for any method - even one which is not expert - to be used without large error.

2. We tried using the dry incineration method as stated in Polish Pharmacopeia IV for testing contamination of heavy metals, where the ash dissolved in 0.1n HCl heated for 5 mins. in a water bath.

In the assay of tin, this method produced a 100% error. In products to which known quantities of standard tin solution were added, there was generally no tin discovered.

3. Another method was also tried (suggested for the dry mineralization of organic substances [7]). In comparison with the previous method, this method turned out better; nevertheless, excess tin constituted about 20% since the addition of 65% HNO_3 made the reaction very turbulent, causing the product to disintegrate. For this reason, ash dissolved in 10% HCl produced much

better results in comparison with the previous method.

4. It was thus decided to mineralize samples having the same tin content as before (as in point 1 of the experimental section); ash from some samples was dissolved in 10%, from some samples in HCl with a (1 + 1) concentration, and others in acids having the same concentrations as above, but pre-heated in steam chambers 10 min. in boiling water. In samples having cold-dissolved ash, the tin remainder constituted about 15%, while in samples having hot-dissolved ash, much better results were produced, since the error constituted zero percent.

5. Therefore, we repeated the experiment with tomato past, banana juice, banana compote, and herring in oil. Six samples of each product were selected for mineralization: for incineration (as per point 1 of the experimental section), three samples of each product were dissolved in 10% HCl (heated) and three in HCl (1 + 1) (also heated) for 10 min. in a water bath. In carrying out the reaction with dithiol and visual comparison of the samples in question with a standard scale, there was no difference between the quantity of added tin in the products and the computed quantity.

The results of assaying tin content in these samples was effected by using a photocolorimeter (Spekol) which has significantly greater precision than visual comparison; these results are shown in Table 2. As can be seen from the applied table, error is small for this procedural method (no exceeding 5%). Moreover, no difference in the results of tin content assay was noted for samples having ash dissolved in HCl(1 + 1) or in 10% HCl.

Also conducted was a test for samples in which the ash was dissolved in HCl (1 + 1) with an addition of 0.5 HCl (1 + 1) (for tin assay by the dithiol method). For this purpose, to a series of samples were pipetted 1, 2, 3, and 4 ml of the solution in question, supplemented by water (5 ml) and 0.5 ml HCl (1 + 1); for another series of samples, the same amounts of the same filtrate were added, supplemented by water to make 5.5 ml, without adding any HCl (1 + 1); a reaction with dithiol was then produced. The tin mercaptide residue was maintained in a state of suspension for the longest time (about 48 min.), between the content of samples to which 0.5ml HCl(1 + 1) had been added and those to which no acid was added. Thus, from this point of view and, bearing in mind the acid concentration according to Bernstein and Gilewska [1] - (0.5 ml

Table 2

Results of Tin Assays in Dry-Mineralized Products with Ash Hot-Dissolved in HCl(1+1) and HCl 10%.

Product	Weight g 2X:	Amount of tin added in coordinated control test, mg/kg	Sn Discovered in ash dissolved, mg/kg		% Error	
			1. in 10% HCl	2. in HCl (1+1)	1	2
Banana compote.....	20	—	0	0	—	—
"	20	50,0	47,50	51,25	—5,0	+2,5
"	20	50,0	48,75	50,00	—2,5	0,0
"	20	50,0	50,00	49,38	0,0	—0,6
Banana juice	20	—	37,50	37,50	—	—
"	20	87,5	87,50	87,50	0,0	0,0
"	20	87,5	87,50	88,75	0,0	+1,4
"	20	87,5	86,25	87,50	—1,4	0,0
tomato paste	20	—	0	0	—	—
"	20	100,0	101,25	105,00	+1,3	+5,0
"	20	100,0	102,50	100,00	+2,5	0,0
"	10	200,0	197,50	200,00	—1,3	0,0
Herring in oil	20	—	0	0	—	—
"	20	200,0	200,00	200,00	0,0	0,0
"	10	200,0	195,00	195,00	—2,5	—2,5
"	10	200,0	200,00	197,50	0,0	—1,3

HCl (1+1) per 5 ml of sample), this was established for samples in which the ash was dissolved in HCl (1+1), without adding HCl, allowing to reduce analysis by one operation.

Using samples which were accessible to the laboratory at the time (tomato paste, pickles, herring in oil) for the purpose of assaying their tin content, it was resolved to perform mineralization via two methods at the same time: wet [8], and dry with ash dissolved in HCl(1+1) in a water bath. The results of these assays are shown in Table 3.

Note: for each assay series, a standard scale was prepared with a solution of tin; this was compared with the solutions in question visually and by colorimeter. Plotting the series of reference curves was done with special attention to standard conditions which must be maintained for colorimetric assay of tin by the dithiol method. The dithiol solution must be freshly prepared from a pure source [1].

Table 3
Results of Tin Assay in Wet and Dry Mineralized Products

Product	Amount of product used for incineration g	Sn Discovered in Mineralization of product, mg/kg		Difference, mg	Difference (%) in results of wet mineralization
		wet	dry		
tomato paste	2×20	190,0	190,0	0	0,0
pickles	2×20	65,0	67,5	2,5	+3,8
herring in oil	2×10	250,0	245,0	5	—2,0

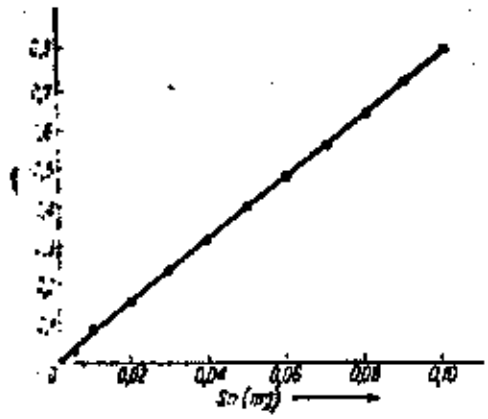


Fig. 1. Reference Curve of Standard Tin Solution - with Fresh Dithiol. Assay performed on SPEKOL spectro-colorimeter (Zeiss Company).

A dithiol solution prepared from an old source (greenish color, temperature less than 35°C) somewhat causes weak intensity of color in addition to more rapid turbiziation and coagulation of sediment; but the standard tin curve plotted with the same solution (graph of extinction as a function of concentration) does not form a straight line. This should be particularly remembered for photocolorimetric assays because it may happen that the tin content findings in the samples in question - when prepared with old dithiol - when read out on a photocolorimeter and compared with the plotted standard curve will sooner be (with fresh dithiol) much greater than actual findings.

In using the read outs on the Model 581 colorimeter, the differences were small; but on the "Spekol",

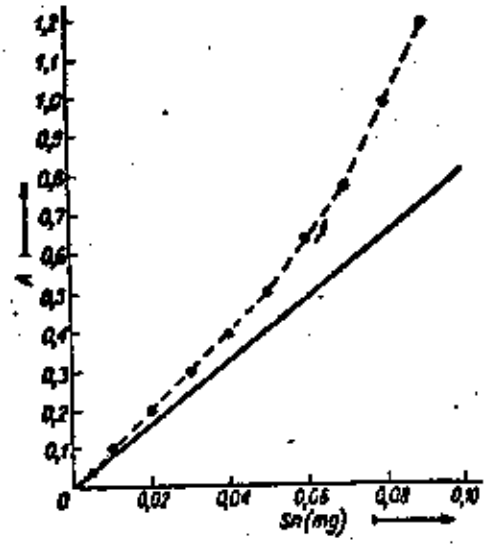


Fig. 2. Reference Curve of Standard Tin Solution - with Old Dithiol.
Assay performed on SPEKOL spectrocolorimeter (Zeiss Company).

which is very sensitive, they were great, as is shown in Figs. 1 & 2.

The standard tin curve shown in Fig. 1 was first prepared with dithiol which had been freshly-prepared and came from a freshly-opened ampoule (curve complies with the Lambert-Beer law). The curve shown in Fig. 2, however, was first prepared with fresh-prepared dithiol solution, but from an ampoule which had been opened three weeks prior (stored at room temperature) with which the dithiol was weighed four times (ampoule sealed with rubber stopper). For this reason, it is best to order an ampoule containing 0.5 or 1g of dithiol which can be partially opened according to the amount

required (to prevent entrance of air prior to opening) and can be stored at room temperature.

The results of the research described in the present paper is designated for incineration of foodstuffs by the dry method and assay of tin with dithiol.

Procedure

A certain weight of uniform, average test sample is weighed in a porcelian steam dish correct within 0.01g and is dried in a dessicator at 100°C. Liquid or semi-liquid products (juices, compotes) are evaporated in advance in a water bath. Then, the contents of the steam dish are dried on an electric plate (on an asbestos mesh), after which they are carefully carbonized over a low burner flame (seeing that the product is not ignited by the flame - be careful!) to complete smoldering. They are then incinerated in a muffle furnace at 450-500°C. The resulting ash is dissolved in 20 ml HCl (1 + 1), heated for 10 min. in boiling water, and mixed from time to time with a glass stirrer (which is inserted into the steam dish when the acid is added).

After cooling, the contents are transfered by quantiy from the steam dish to an ash-free filter; they are filtered into a 100ml measuring flask; the stirrer, steam dish, and filter are washed several times with boiling distilled water. On cooling to 20°C, the flask contents are brought up to 100ml. From the filtrate prepared in this way , we pipette 1, 2, 2.5, or more milliliters into the sample (depending on the expected tin content), adding water to make 5.5ml; three drops of thioglycolic acid are added to reduce Sn^{4+} to SN^{2+}; the preceeding is mixed and let stand for 5-10 min. Then, 0.5ml of Mersapon solution (from a microburette) is added (2ml commercial Mersapon supplemented by distilled water in a measuring flask [100ml]; then mix with rotary action and heat for 3 min. in a boiling water bath. After this, add (using a drop funnel) 5 drops of freshly-prepared dithiol solution (0.2 dithiol and 0.5 thioglycolic acid dissolved in 100ml 1% NaOH) and mix thoroughly with rotary action. Then, compare this with a parallel standard scale from 5 to 50μg Sn; or effect absorption measurement on a photoelectric colorimeter and read out the desired tin content from the reference curve of standard tin with contents ranging from 5-100μg Sn.

In preparing the standard scale, the amounts of tin element, water, acid, and Mersapon solution should be measured out with a microburette.

Conclusions

The mineralization of foodstuffs by the dry method and by dissolving the ash in a water bath containing HCl (1 part HCl + 1 part H_2O) or in 10% concentration possesses sufficient accuracy for assaying the tin content of foodstuffs.

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OLIMPIA GAJEK, HELENA KLIMCZAK

MINERALIZACJA NA SUCHO STOSOWANA PRZY OZNACZANIU CYNY W ŻYWNOŚCI

Z Pracowni Badania Żywności Portowej Stacji Sanitarno-Epidemiologicznej w Gdyni.
Dyrektor: dr *J. Stankiewicz*

Przy badaniu żywności na zawartość metali ciężkich szkodliwych dla zdrowia najczęściej zachodzi konieczność oznaczania zawartości cyny.

Na przykład w produktach żywnościowych przechowywanych w puszkach konserwowych tłoczonych cynowanych nie lakierowanych albo z lakierem uszkodzonym, nie ma właściwie potrzeby oznaczania ołowiu, miedzi czy cynku, lecz przede wszystkim cyny. W żywności przechowywanej w puszkach konserwowych składanych, w których nie ma przecieków lutu do wewnątrz lub w których próbą jakościową na ołów stwierdzono nikły jego ślad w górnej lub dolnej części szwu płaszcza, również przeprowadzamy analizę na cynę, gdyż — jak wykazała praktyka — ołowiu w produkcie nie ma wcale albo jest najwyżej ślad.

Dotychczas obowiązujące nas przepisy (Polskie Normy, Podręczniki Analizy Żywności) przy oznaczaniu metali ciężkich w żywności — z wyjątkiem cynku — podają metodę mineralizacji na mokro.

Wiadomo, że mineralizacja na mokro trwa stosunkowo długo (szczególnie przy takich produktach, jak ryby czy mięso), zużywa znaczne ilości odczynników cz.d.a. oraz — co najważniejsze ze względu na wydzielające się tlenki azotu szkodliwe dla zdrowia — wymaga dobrego wyciągu lub osobnego pomieszczenia, — co nie zawsze leży w możliwościach technicznych każdej mniejszej pracowni kontroli żywności.

Ponieważ w pracowniach badania żywności najczęściej oznacza się zawartość cyny — o czym była mowa wyżej — podjęto więc próbę zastąpienia mineralizacji na mokro taką metodą mineralizacji na sucho, której straty cyny będą najmniejsze.

W tym celu spalono szereg różnych produktów żywnościowych, do których dodawano określone ilości roztworu wzorcowego cyny i po rozpuszczeniu popiołu oznaczano zawartość cyny metodą ditiolową [1] przy użyciu detergenta o nazwie „MERSAPON" [2]. Popiół rozpuszczano różnymi sposobami, które zostały omówione w części doświadczalnej niniejszej pracy.

Do każdej serii oznaczeń przygotowywano skalę wzorców z roztworem cyny, z którą porównywano badane próby wizualnie i za pomocą fotokolorymetru produkcji chińskiej model 581, posługując się filtrem zielonym, lub SPEKOL-u firmy Zeiss przy długości fali 535 mμ [3].

CZĘŚĆ DOŚWIADCZALNA

1. Do pasty pomidorowej, w której stwierdzono uprzednio, że nie zawiera cyny, dodano określoną ilość roztworu wzorcowego cyny i po dokładnym wymie-

szaniu w moździerzu odważono do parowniczek porcelanowych próbki 10- i 20-gramowe i wstawiono do suszarki o temp. 100° (na okres ok. 4 godz.), a następnie podsuszano dalej na płytce elektrycznej (na siatce azbestowej), po czym ostrożnie zwęglano małym płomieniem palnika aż do całkowitego wydymienia, a następnie spopielano w piecu elektrycznym w temp. 450 — 500°.

Otrzymany popiół (barwy szarej) przenoszono ostrożnie do tygielka żelaznego, na którego dno dawano 5 — 8 granulek (w zależności od ilości popiołu) ługu sodowego [4] i stapiano na palniku (przeprowadzając cynę w cyniany rozpuszczalne w wodzie) [5].

Po ostygnięciu dodawano do tygla niewielką ilość (ok. 5 ml) wody destylowanej, ostrożnie podgrzewano, po czym przenoszono zawartość tygla (ilościowo) na sączek bezpopiołowy i sączono do kolby miarowej na 100 ml, popłukując kilkakrotnie tygiel i sączek gorącą wodą do ok. $^2/_3$ objętości kolbki.

Następnie do kolbki z przesączonym roztworem dodawano 2 krople roztworu fenoloftaleiny i zakwaszono kwasem solnym do całkowitego odbarwienia (cyniany przeprowadzano w chlorek cynowy). Po ochłodzeniu zawartości kolbki do temp. 20° uzupełniano ją wodą destylowaną do obj. 100 ml. Z tak przygotowanego roztworu pobierano pipetą 1 lub 2 ml (w zależności od dodanej ilości cyny) i oznaczano zawartość cyny metodą ditiolową [1].

W ten sam sposób przeprowadzono doświadczenie z próbami kompotu ananasowego i śledzia w oleju. Przy porównaniu wizualnym badanych prób ze skalą wzorców, tylko w niektórych probówkach zauważono bardzo niewielkie (nie dające się dokładnie określić) różnice w natężeniu barwy czerwonego merkaptydu cyny. Różnice te odczytane dokładnie za pomocą fotokolorymetru podaje tab. I.

Tabela I

Wyniki oznaczania cyny w produktach zmineralizowanych na sucho i stopionych z ługiem

Nazwa produktu	Ilość g konserwy użytej do spalenia	Dodano mg Sn na próbkę	Ilość mg Sn razem ze ślepą próbą w przeliczeniu na 1 kg	Wykryto mg Sn na 1 kg	% błędu
Pasta pomidorowa	20	—	—	0	—
" "	20	4	200	200,0	0,0
" "	10	2	200	185,0	—7,5
" "	10	2	200	195,0	—2,5
Kompot ananasowy	20	—	—	42,5	—
" "	20	1	92,5	85,0	—8,1
" "	20	1	92,5	87,5	—5,4
" "	20	1	92,5	88,7	—3,8
Śledź w oleju	20	—	—	0	—
" "	20	4	200	185,0	—7,5
" "	20	4	200	205,0	+2,5
" "	10	2	200	181,6	—9,2

Jak widać z tabeli wyniki otrzymano dość dobre — błąd poniżej 10%. Ale żeby dojść do takich wyników, trzeba było wykonać szereg prób, aby poznać i ulepszyć słabe strony tej metody, która wymaga dość dużo czynności, jak: przenoszenie

popiołu z parowniczki do tygielka, stapianie z ługiem, ilościowe przenoszenie zawartości tygla do kolbki, zakwaszanie do odpowiedniego pH, aby nie powstała opalizacja czy osad (w czym bardzo nam pomógł dodatek fenoloftaleiny) itd.

Przy tym jeżeli popiół zawiera ślady produktu zwęglonego (nie spopielonego), to po stopieniu z ługiem powstaje barwa brunatna, przechodząca przez sączek i próba taka już nie nadaje się do oznaczeń kolorymetrycznych. Szukano więc metody, którą mógłby każdy — nawet mało wprawny analityk — posługiwać się bez większego błędu.

2. Próbowano zastosować metodę spalania na sucho podaną w Farmakopei Polskiej IV przy próbie na zanieczyszczenia metalami ciężkimi, w której popiół rozpuszcza się w 0,1 n HCl ogrzewając przez 5 min. na łaźni wodnej [6].

Metoda ta, w wypadku oznaczania cyny, dała 100% błędu. W produktach, do których dodawano wiadome ilości roztworu wzorcowego cyny, w ogóle cyny nie wykryto.

3. Wypróbowano również drugą metodę (podaną przy mineralizacji na sucho substancji organicznych) [7]. Ta metoda w porównaniu z poprzednią okazała się lepsza, jednakże straty cyny wynosiły ok. 20%, gdyż dodatek 65% HNO_3 czynił reakcję bardzo burzliwą, co powodowało pryskanie produktu. Jednakże rozpuszczenie popiołu w 10%-owym HCl dawało, w porównaniu z metodą poprzednią, dużo lepsze wyniki.

4. Postanowiono więc zmineralizować próby z taką samą zawartością cyny jak poprzednio, (jak w punkcie 1 części doświadczalnej) i rozpuszczono popiół części prób w 10%-owym HCl i części prób w kwasie solnym o stężeniu (1 + 1), oraz części prób w kwasach o takim samym stężeniu jak wyżej, ale ogrzewając parowniczki przez 10 min. na wrzącej łaźni wodnej. W próbach z popiołem rozpuszczonym na zimno niedobór cyny wynosił ok. 15%, a w próbach z popiołem rozpuszczonym na ciepło wyniki uzyskano bardzo dobre, gdyż błąd wynosił zero procent.

5. Powtórzono więc jeszcze raz to doświadczenie z pastą pomidorową, sokiem ananasowym, kompotem bananowym i śledziem w oleju. Do mineralizacji brano po 6 prób z każdego produktu: po spopieleniu (wg pkt. 1 części doświadczalnej) rozpuszczano po 3 próby (z każdego produktu) w 10%-owym HCl na ciepło i po 3 w HCl (1 + 1) również ogrzewając przez 10 min. na łaźni wodnej. Po przeprowadzeniu reakcji z ditiolem i porównaniu wizualnym badanych prób, z równocześnie skalą wzorców, nie stwierdzono żadnej różnicy pomiędzy ilością cyny dodaną do badanych produktów a odczytaną.

Wyniki oznaczeń zawartości cyny w tych próbach dokonano za pomocą fotokolorymetru („Spekol"), który daje znacznie większą dokładność aniżeli porównanie wizualne, przedstawia tab. II. Jak widać z załączonej tabeli, przy tym sposobie postępowania, błąd jest mały (nie przekracza 5%). Przy tym nie zauważono żadnej różnicy w wynikach oznaczania zawartości cyny w próbach z popiołem rozpuszczonym w kwasie solnym (1 + 1) lub w kwasie solnym 10%-owym.

Sprawdzono również, czy do prób, w których popiół był rozpuszczony w HCl (1 + 1) konieczne jest jeszcze dodawanie (przy oznaczaniu cyny metodą ditiolową) 0,5 ml HCl (1 + 1). W tym celu do szeregu probówek odpipetowano po 1, 2, 3 i 4 ml badanego roztworu, uzupełniono do 5 ml wodą i dodano po 0,5 ml HCl (1 + 1), drugi szereg probówek, do którego odmierzono takie same ilości tego samego przesączu, uzupełniono wodą do 5,5 ml nie dodając już HCl (1 + 1) i przeprowadzono reakcję z ditiolem. Pomiędzy zawartością probówek, do których dodano po 0,5 ml HCl (1 + 1), a tymi do których kwasu nie dodano, nie stwierdzono żadnej różnicy w barwie i klarowności. W probówkach, do których kwasu nie dodano, osad merkaptydu cyny utrzymywał się w stanie rozproszenia

najdłużej (około 48 godz.). Z tego więc względu, jak również biorąc pod uwagę stężenie kwasu podane przez *Bernstein* i *Gilewską* [1] — (0,5 ml HCl (1 + 1) na 5 ml próby) postanowiono do prób, w których popiół był rozpuszczony w HCl (1 + 1), już nie dodawać kwasu solnego, co pozwoli skrócić analizę o jedną czynność.

Tabela II

Wyniki oznaczenia cyny w produktach zmineralizowanych na sucho i po rozpuszczeniu popiołu na ciepło w kwasie solnym 1+1 i 10%-owym

Nazwa produktu	Wielkość naważki w g 2 × po:	Ilość dodanej cyny łącznie ze ślepą próbą w mg/kg	Wykryto Sn w mg/kg po rozpuszczeniu popiołu		% błędu	
			1) w HCL 10%-owym	2) w HCl 1+1	ad 1	ad 2
Kompot bananowy	20	—	0	0	—	—
Kompot bananowy	20	50,0	47,50	51,25	—5,0	+2,5
Kompot bananowy	20	50,0	48,75	50,00	—2,5	0,0
Kompot bananowy	20	50,0	50,00	49,38	0,0	—0,6
Sok ananasowy	20	—	37,50	37,50	—	—
„ „	20	87,5	87,50	87,50	0,0	0,0
„ „	20	87,5	87,50	88,75	0,0	+1,4
„ „	20	87,5	86,25	87,50	—1,4	0,0
Pasta pomidorowa	20	—	0	0	—	—
Pasta pomidorowa	20	100,0	101,25	105,00	+1,3	+5,0
Pasta pomidorowa	20	100,0	102,50	100,00	+2,5	0,0
Pasta pomidorowa	10	200,0	197,50	200,00	—1,3	0,0
Śledź w oleju	20	—	0	0	—	—
„ „	20	200,0	200,00	200,00	0,0	0,0
„ „	10	200,0	195,00	195,00	—2,5	—2,5
„ „	10	200,0	200,00	197,50	0,0	—1,3

Korzystając z prób, które dostarczono w tym czasie do laboratorium (pasta pomidorowa, ogórki konserwowe, śledzie w oleju) w celu oznaczenia w nich zawartości cyny, postanowiono przeprowadzić mineralizację równolegle dwoma sposobami: na mokro [6] i na sucho z rozpuszczeniem popiołu w HCl (1 + 1) na łaźni wodnej. Wyniki tych oznaczeń przedstawia tab. III.

Uwaga. Do każdej serii oznaczeń przygotowywano równocześnie skalę wzorców z roztworem cyny, z którą porównywano badane roztwory wizualnie i fotokolorymetrycznie. Wykreślając szereg krzywych kalibracji zwrócono szczególną uwagę na podstawowy warunek, który musi być zachowany przy kolorymetrycznym oznaczaniu cyny metodą ditiolową. Roztwór ditiolu musi być świeżo przyrządzony z czystego odczynnika [1].

Tabela III

Wyniki oznaczania cyny w produktach zmineralizowanych na mokro i na sucho

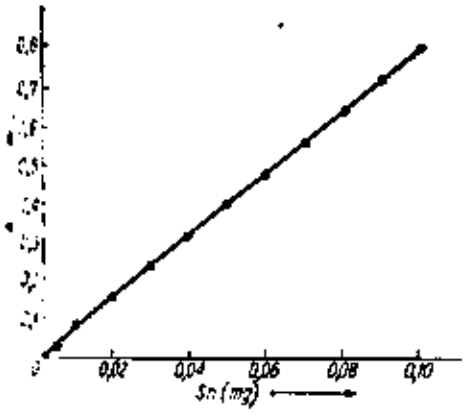
Nazwa produktu	Ilość g produktu użyta do spalenia	Wykryto Sn w mg/kg po mineralizacji produktu		Różnica w mg	Różnica (w procentach) wyniku mineralizacji na mokro
		na mokro	na sucho		
Pasta pomidorowa	2×20	190,0	190,0	0	0,0
Śpinki konserwowe	2×20	65,0	67,5	2,5	+3,8
Śledź w oleju	2×10	250,0	245,0	5	—2,0

Roztwór ditiolu przyrządzony z nieświeżego odczynnika (barwa żółta, temp. tpn. poniżej 35°) nie tylko powoduje słabsze natężenie barwy oraz szybsze zmętnienie i koagulację osadu, lecz krzywa wzorcowa cyny wykreślona z tym roztworem (wykres zależności ekstynkcji od stężenia) nie stanowi linii prostej. Należy o tym szczególnie pamiętać przy oznaczeniach fotokolorymetrycznych, gdyż może się zdarzyć, że wyniki zawartości cyny w próbach badanych — przyrządzonych z ditiolem nieświeżym — odczytane na fotokolorymetrze i porównane z krzywą wzorcową wykreśloną wcześniej (z ditiolem świeżym), będą znacznie wyższe od rzeczywistych.

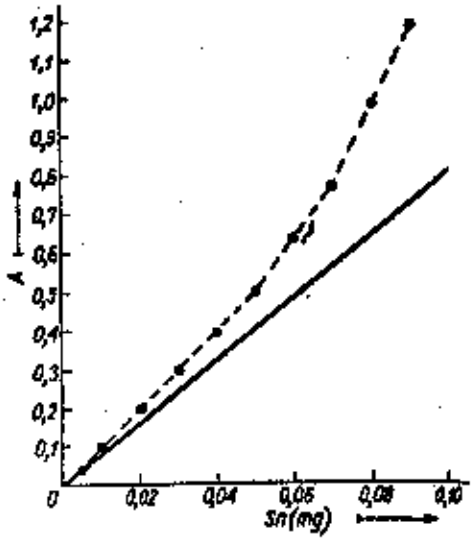
Dokonując odczytów na kolorymetrze model 581, różnice zauważono niewielkie, jednakże na „Spekolu", który jest bardzo czuły, były one duże, co obrazują rys. 1 i 2.

Krzywa wzorcowa cyny przedstawiona na ryc. 1 została wykonana z roztworem ditiolu świeżo przyrządzonym i ze świeżo otwartej ampułki (krzywa podlega prawu *Lamberta — Beera*). Natomiast krzywa przedstawiona na ryc. 2 została wykonana z roztworem ditiolu świeżo przyrządzonym, lecz z ampułki otwartej przed trzema tygodniami (przechowywanej w lodówce), z której ditiol był odważony już czterokrotnie (ampułka zamykana gumowym koreczkiem). Dlatego najlepiej jest zamawiać ampułki z ditiolem 0,5- lub 1-gramowe, które po odważeniu potrzebnej ilości ditiolu należy natychmiast szczelnie zamknąć (aby zabezpieczyć przed dostępem powietrza) i przechowywać w lodówce.

W wyniku naszych doświadczeń opisanych w niniejszej pracy, podajemy przepis na spalenie żywności metodą na sucho i oznaczanie cyny ditiolem.



Ryc. 1. Krzywa kalibracji roztworu wzorcowego cyny — z ditiolem świeżym. Oznaczenie przeprowadzono na spektrokolorymetrze SPEKOL firmy Zeiss.



Ryc. 2. Krzywa kalibracji roztworu wzorcowego cyny — z ditiolem niedwiedzym. Oznaczenie przeprowadzono na spektrokolorymetrze SPEKOL firmy Zeiss.

SPOSÓB POSTĘPOWANIA

Odważkę jednorodnej, średniej próbki laboratoryjnej odważyć w parowniczce porcelanowej z dokładnością do 0.01 g i podsuszyć w suszarce w temp. 100°. Produkty płynne lub półpłynne (soki, kompoty) odparować uprzednio na łaźni wodnej. Następnie zawartość parowniczki dosuszyć na płytce elektrycznej (na siatce azbestowej), po czym ostrożnie zwęglić małym płomieniem palnika (uważając, aby produkt nie zapalił się płomieniem — b. ważne!) aż do całkowitego wydymienia, a następnie spopielić w piecu elektrycznym w temp. 450 — 500°. Otrzymany popiół rozpuścić w 20 ml HCl (1 + 1), ogrzewając w ciągu 10 min. na wrzącej łaźni wodnej i mieszając od czasu do czasu bagietką szklaną (włożoną do parowniczki w momencie dodawania kwasu).

Po ochłodzeniu przenieść ilościowo zawartość parowniczki na sączek bezpopiołowy i przesączyć do kolby miarowej poj. 100 ml, poplukując bagietkę, parowniczkę i sączek kilkakrotnie gorącą wodą destylowaną. Po ochłodzeniu do temp. 20° uzupełnić zawartość kolbki do 100 ml. Z tak przygotowanego przesączu odpipetować do probówki 1, 2, 2,5 ml itd. (w zależności od spodziewanej zawartości cyny) i uzupełnić wodą (z mikrobiurety) do 5,5 ml, dodać 3 krople kwasu tioglikolowego (aby zredukować Sn^{4+} do Sn^{2+}), zamieszać i odstawić na 5 — 10 min. Następnie dodać 0,5 ml (z mikrobiurety) roztworu mersaponu (2 ml mersaponu handlowego uzupełnione wodą destylowaną w kolbie miarowej do 100 ml), zamieszać ruchem wirowym i ogrzewać 3 min. we wrzącej łaźni wodnej, po czym natychmiast dodać (za pomocą wkraplacza) 5 kropel świeżo przyrządzonego roztworu ditiolu (0,2 g ditiolu i 0,5 g kwasu tioglikolowego rozpuścić/ w 100 ml 1%-owego NaOH) zamieszać dokładnie ruchem wirowym i porównać z przygotowaną równocześnie skalą wzorców od 5 do 50 µg Sn, albo dokonać pomiaru absorbcji na kolorymetrze fotoelektrycznym i odczytać szukaną zawartość cyny z krzywej kalibracji roztworu wzorcowego cyny o zawartości od 5 — 100 µg Sn.

Przygotowując skalę wzorców należy ilości roztworu wzorcowego cyny, wodę, kwas i roztwór mersaponu odmierzać za pomocą mikrobiuret.

WNIOSKI

Mineralizacja artykułów żywnościowych metodą na sucho i rozpuszczenie popiołu na łaźni wodnej w kwasie solnym o stężeniu (1 + 1) lub 10%-owym, daje wystarczającą dokładność przy oznaczaniu zawartości cyny w żywności.

O. Gajek, H. Klimczak

МЕТОД СУХОЙ МИНЕРАЛИЗАЦИИ ПРИМЕНЯЕМЫЙ ПРИ ОПРЕДЕЛЕНИИ ОЛОВА В ПИЩЕВЫХ ПРОДУКТАХ

Содержание

Разработан метод сухой минерализации применяемой при обозначении олова в пищевых продуктах. Полученная зола растворяется в 20 мл 10%-ой соляной кислоты или концентрации 1 часть воды + 1 часть HCl. В растворе определяют содержание олова дитиолевым методом, употребляя 0,5 мл детергента — мерсапон.

Кривая калибрации вычеркнутая для градировки шкалы от 0 до 100 с добавкой мерсапона подчиняется закону Lamberta-Beera.

O. Gajek, H. Klimczak

A METHOD OF DRY MINERALIZATION FOR THE TIN DETERMINATION IN FOOD

Summary

A method of dry mineralization for the determination of tin in food has been developed.

The ashes obtained were dissolved in 20 ml of 10% HCl, or in that 1 + 1. In the filtrates, tin was assayed by the dithiol method 0.5 ml of the detergent "mersapon" being added.

The reference curve, determined from the range of standards from 0 to 100 mg of Sn, has proved to meet the Lambert — Beer law.

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EFFECT OF STANNOUS CHLORIDE ON THE COLOR OF GLASS-PACKED KRAUT

by J. R. Geisman, Associate Professor

Taken from Research Progress Reports, Department of Horticulture, Ohio State University

ONE PROBLEM frequently encountered in sauerkraut packaged in glass containers is the discoloration due to exposure to light. This reaction occurs at a relatively slow rate with the end results of downgrading the quality of the product. This study was undertaken to determine whether stannous chloride could be added to sauerkraut packed in glass to prevent discoloration.

The addition of stannous chloride to asparagus is permitted by the Food and Drug Administration. The maximum content allowable was 15 ppm. The amounts added to sauerkraut for this experiment varied from 0 to 15 ppm. by 2.5 ppm. increments.

A standard procedure was utilized in filling, closing and storing. The acidity of the kraut was determined and standardized at 1.0 percent as lactic. The salt content was kept at 1.3 percent.

Kraut was heated to 165 degrees F. and twelve ounces were filled in pint glass jars. The proper amount of stannous chloride was added. Hot brine (180 degrees F.) was added to cover the shreds; appromixately four ounces. The jars were steam-flow closed and allowed to cool.

The jars were placed at room temperature uncased for exposure to light. Visual observations were made at weekly intervals on the uniformity of color. Samples were opened after one, three and six months storage. At these times, pH, total acid, and salt determinations were made.

No changes in any of these attributes were noted during storage. In addition, objective color measurements were made utilizing the Agtron F color instrument. These data were compared to similar data obtained prior to packaging the kraut and are presented in Table I. This instrument was standardized at 30 on a gray disc (Monsanto Lustrex 5019.5) and at zero on a black disc (Monsanto Lustrex 00).

Since as Agtron values decrease, the color darkens, the data indicated that the kraut darkened throughout the storage period. Kraut with no stannous chloride added darkened considerably and was unacceptable after three months storage.

The least change in Agtron color value occurred with the samples containing 15 ppm stannous chloride. However, by visually observing the samples, it was noted that samples containing more than 5 ppm stannous chloride were not uniform in color. A precipitate was formed which turned some shreds black and there were definite light and dark layers within the package making the product unwholesome in appearance. Further investigations are underway to determine whether this objection can be overcome.

Concentrations less than five part per million offer some promise in delaying or retarding darkening. This aspect is also under further investigation.

TABLE I. AGTRON COLOR VALUES FOR SAUERKRAUT STORED FOR 1, 3 AND 6 MONTHS CONTAINING VARIOUS CONCENTRATIONS OF STANNOUS CHLORIDE

Stannous Chloride (ppm)	Agtron F Color Values At			
	0 Month	1 Month	3 Months	6 Months
0.0	47	45	26	22
2.5		45	33	27
5.0		47	39	29
7.5		45	37	29
10.0		45	37	29
12.5		45	37	32
15.0		45	36	34

SHORT COMMUNICATIONS

An Improvement in the Method of Ashing of Canned Foods for the Estimation of Tin

A method involving dry ashing along with a mixture of potassium dihydrogen phosphate and magnesium nitrate is described. Ashing is quick with no volatilization of tin. The method is simple, precise, requires minimum of precautions and is satisfactory for the routine estimation of tin in canned foods.

In canned foods, tin dissolves into the foods due to the interaction between the container and the contents and tin content gives an indication of the extent of corrosion of the container and the acceptability of the contents. Estimation of tin in any canned product assumes importance in assessing the quality of the food product.

Existing methods for the estimation of tin are based on (*i*) gravimetric, (*ii*) spectrographic, (*iii*) polarographic, (*iv*) colorimetric and (*v*) volumetric methods. Among these, modified colorimetric method[1] originally developed by Clark[2,3] and volumetric method by Mckenzie[4] are widely in use. Mckenzie's method involves destruction of the organic matter by wet digestion. This is time consuming, and requires a fuming chamber which needs very frequent servicing because of the nitric fumes which attack the fan. Clark's method involves dry ashing of the sample and development of the colour using dithiol. This method suffers from two serious disadvantages (*i*) loss of metal due to volatilisation and fusion with the silica of the dish during ashing and (*ii*) interference due to copper which has to be removed before the colour development.

The present study was undertaken to develop a simple reliable and accurate method of dry ashing (by overcoming volatilization and attack on the silica dish) and estimation of tin by volumetric procedure. The following compounds were tried by incorporating them in the material before ashing: (*i*) tripotassium phosphate, (*ii*) potassium dihydrogen phosphate (*iii*) potassium monohydrogen phosphate (*iv*) trisodium phosphate (*v*) sodium dihydrogen phosphate (*vi*) sodium monohydrogen phosphate (*vii*) magnesium nitrate, (*viii*) magnesium acetate (*ix*) calcium nitrate and (*x*) calcium acetate. Among these, potassium and sodium dihydrogen phosphates and magnesium nitrate were found to eliminate almost completely the loss of tin during ashing and gave very satisfactory results. However, with the phosphates, ashing was extremely slow even at 550° C. It had to be completed by moistening with nitric acid and later removing the excess nitric acid by adding hydrogen peroxide and boiling.

Ashing was very quick with magnesium nitrate and white ash was obtained within 2 hr at 550°C. However, there was every possibility of fine particles of ash escaping within the muffle because of the extreme lightness. Hence, a mixture of magnesium nitrate and potassium dihydrogen phosphate was tried with excellent results. The process of ashing was very quick and loss of ash due to lightness of particles was practically eliminated.

The process adopted with canned foods was as follows:

The semi solid canned foods were homogenized in the Waring blendor while the liquid foods were mixed to get a representative sample. Fifty grams of the homogenized sample was taken in a silica dish. Two grams each of potassium dihydrogen phosphate and magnesium nitrate were added and mixed well with a few milli litre of distilled water. It was evaporated on a water bath and when dry the product was charred on a bunsen burner. The charred product was incinerated in a muffle furnace at 550° C for 3–4 hr. and cooled. Five to 10 ml of hydrogen peroxide was added and the contents boiled over a burner to remove the nitrous fumes. Concentrated hydrochloric acid (5 ml) was added and evaporated on the water bath and 5 ml of this acid was again added and the solution made upto 100 ml with distilled water. An aliquot was reduced by aluminium and titrated with N/200 KIO_3[4]. The tin content was calculated from the titre value.

Reproducibility and accuracy of results by this procedure, were tested on standard tin solutions (in 3N. HCl) prepared from AR tin and AR stannous chloride and by conducting recovery tests. Analar starch (0.5 g) was used to give false ash with standard

TABLE 1. VALUES OF TIN BY WET DIGESTION AND ASHING METHODS IN CANNED PRODUCTS AND IN PURE TIN SOLUTIONS

Product	Wet digestion	Dry ashing with KH_2PO_4	Dry ashing with $Mg(NO_3)_2.2H_2O$	Dry ashing with the mixture of $Mg(NO_3)_2.H_2O$ and KH_2PO_4	Dry ashing
	Tin in ppm*				
Tomato juice	97.0	96.5	92.6	98.8	26.5
Green peas in brine	155.0	155.5	155.0	155.0	46.6
Cabbage in brine	210.4	209.8	206.2	209.9	50.2
Potatoes in brine	227.0	226.5	221.5	228.9	56.6
Orange juice	709.0	711.0	708.5	710.9	213.3
Mango nectar	724.0	723.5	720.5	730.0	217.0
Pure tin solution:	Tin in milligrams				
2 mg	1.98 (99.0)	2.00 (100.0)	1.92 (96.0)	3.05 (102.0)	0.66 (33.0)
5.0 mg	4.95 (99.0)	5.00 (100.0)	4.85 (97.0)	5.10 (102.0)	1.62 (32.4)
10.0 mg	9.75 (97.5)	9.90 (99.0)	9.95 (99.5)	9.90 (99.0)	3.10 (31.0)
15.0 mg	14.69 (97.9)	14.85 (99.0)	14.58 (97.21)	14.95 (99.7)	4.02 (26.8)

Figures in parenthesis indicate the % recovery of tin; *Mean of 6 determinations.

solutions. Varying quantities (0.5–4.0 g) of potassium dihydrogen-phosphate and magnesium nitrate were tried along with standard tin solutions and with foods containing known amounts of tin. Two grams of phosphate and 2 g of magnesium nitrate were found to be adequate.

Comparative results of estimation of tin by wet digestion and ashing methods (potassium dihydrogen phosphate and magnesium nitrate, each of them alone and in mixture) are given in Table 1. A perusal of the results indicates that this ashing procedure is as accurate as the wet digestion procedure.

The authors wish to express their gratitude to Dr H. A. B. Parpia, Director of this Institute for his keen interest in this investigation and are also grateful to Mr K. Sebastian for useful discussions during the course of this investigation.

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Acid Dissolution Rate of Sound and White-Spot Enamel Treated with Tin(II) and Fluoride Compounds

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The inhibition or prevention of enamel dissolution in acid has been the basis for many studies used to search for and investigate potential anticariogenic agents. The interest in this procedure has arisen from the endogenic theory of decay[1] and the success of fluoride in reducing dental decay.[2,3] Volker,[4] utilizing a measurement of the weight loss of powdered enamel resulting from exposure to acid, has demonstrated that the acid solubility of enamel is reduced by treating enamel with sodium fluoride. Buonocore and Bibby[5] have adapted this method to a standard procedure which has been widely used to examine a variety of materials.[6] Since the work of Volker[4] and Buonocore and Bibby,[5] experimental procedures for investigating the effect of different materials on the acid solubility of enamel have been varied by using powdered,[6] intact,[7,8] or white spot enamel;[9] treating in vivo[10] or in vitro[9] prior to the acid exposure; conducting the solubility test in vivo[11,12] or in vitro[9] and measuring the solubility differences by weight loss, by analyses for phosphate or calcium[10,13] from the dissolved enamel, by following changes in strength of the acid,[11] by observation of surface changes of enamel,[14] or by microscopic observations of alterations in subsurface enamel.[15]

In general, for any one particular study of the inhibition of enamel solubility, a single set of experimental conditions has been established that has been determined by the requirements for a detectable level of activity and a feasible operational method. A single set of conditions, however, is not likely to be capable of producing a proper evaluation of all materials, particularly if the correlation with in vivo performance is not direct in principle as well as empirically. Thus different investigators using justifiable experimental methods often have obtained contradictory results.[2,10] The limitation of the simple solubility test has been partially compensated for by carrying out a series of exposures of the treated enamel to acid to determine the "life of the protection."[9] In a few studies the entire solubility curve for enamel treated with sodium fluoride has been determined, and the enamel treated with sodium fluoride has always been less soluble than untreated enamel at comparable exposure times over the entire exposure period.[1,16] This latter method, of course, is the complete and proper procedure for evaluation of solubility behavior.

In the present investigation the enamel dissolution rate in acid was measured for enamel (both sound and with an incipient caries-like lesion) treated with stannous fluoride (SnF_2), stannous chloride ($SnCl_2$), and sodium fluoride (NaF), using both a differential and an integral method. Preparatory to a study of treated enamel, the factors affecting the dissolution of enamel have been examined.[17] The rate of dissolution has been found to increase with increasing hydrogen ion and buffer concentration and decreasing buffer strength (i.e., dissociation constant), and to be inhibited by the presence of the reaction products, calcium and phosphate, as well as most cations and a few anions such as fluoride. Also, little difference has been found in the initial solubility rate among different enamel samples.

Materials and Methods

The essential steps of the experimental procedure consisted of treating enamel with stannous fluoride, stannous chloride, or sodium fluoride and then measuring the rate

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of enamel dissolution. In the differential method, the treated enamel was repeatedly exposed for short intervals to several identical batches of an acid solution. In the integral method the treated enamel was exposed to a single acid solution for long periods, approaching equilibrium. The amount of enamel dissolved was determined by chemically analyzing the acid solution for phosphate and, occasionally, for calcium. The influence of the condition of enamel on these measurements was studied by using normal sound enamel and enamel which had been decalcified in vitro to simulate an incipient carious lesion (i.e., white-spot).

The procedure for measuring enamel dissolution rate is similar to that used to study the kinetics of enamel dissolution.[19] Enamel squares were cut with a sectioning saw[20] from human teeth and cemented* to the ends of plastic rods (¼-inch diameter). The teeth were selected at random from sound or nearly sound maxillary central or lateral incisors extracted by local dentists and stored in 3 per cent formaldehyde solution adjusted to pH 7.5. All sides of the enamel squares except the surface were covered with the plastic cement.* The enamel surface was stripped of debris, and the outer layer of enamel was removed by two short rinses in 6 N HCl, followed by a grinding and polishing with silicon carbide abrasives on wet billiard felt.

Both the treatments and the acid exposures were carried out with the same equipment (Fig. 1). The plastic rod supporting the enamel sample was placed in the chuck of a motor (1,725 rpm), and the enamel was immersed in 2 ml. of solution contained in a small plastic cup in a constant-temperature water bath (37° C.). A blanket of moist nitrogen was used to prevent evaporation and, in the case of $Sn^{2}H$ solutions, oxidation. The motor was oper-

* Durabase, Reliance Dental Manufacturing Company, Chicago, Ill.

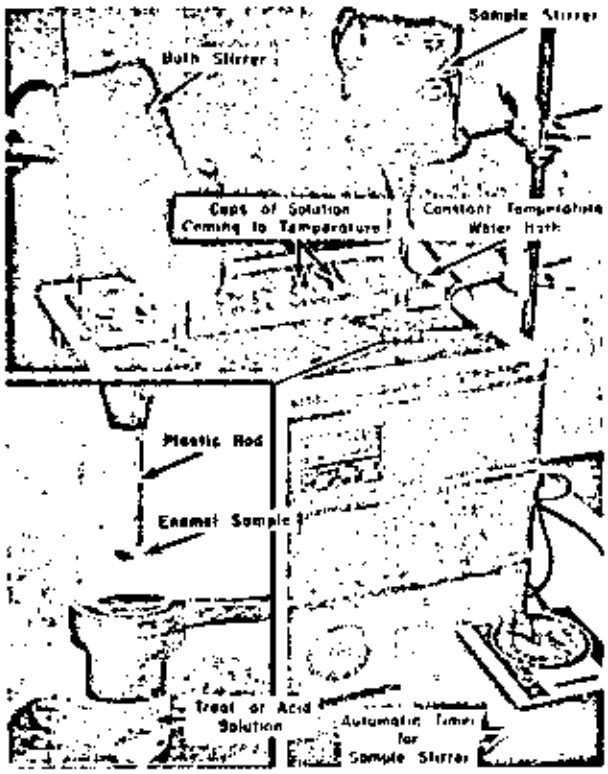

FIG. 1. The apparatus used to determine the solubility rate of enamel treated with various Sn(II) and fluoride compounds is shown along with an exploded view of the enamel sample and solution container. A fin to prevent vortex formation is fixed inside to the bottom of the cup.

tol for the desired interval after which the enamel sample was removed. After a treatment (5-minute treatments were used throughout these experiments), the enamel was rinsed with distilled water before exposure to acid. In the integral method the enamel was left in the acid for the full duration of the experimental period, and the experiment was repeated completely for each time interval. In the differential method the enamel was removed after a short time (2 minutes) and a fresh sample of acid was substituted. Several repeated exposures were so performed, and the results of the separate exposures were summed. All acid solutions were analyzed chemically for phosphate[9] and occasionally for calcium.[10]

The subsurface decalcification of enamel to simulate an incipient carious lesion was made by exposing the sample after polishing and prior to treatment, for 72 hours to an acid solution containing 0.04 M lactic acid.[11] After the exposure the enamel was rinsed with water and lightly polished with abrasive (600) grit on wet billiard felt to remove remnants of the agar medium. The enamel was then handled in the same manner as described in the preceding paragraph.

All treatments were 5 minutes, using approximately 3,000 ppm Sn(II) and or 950 ppm fluoride at the natural pH, which is 3.4 for stannous fluoride, 2.2 for stannous chloride, and 7.0 for sodium fluoride. The conditions were selected as being favorable, practical, and safe for the use of these materials. One of the treating solutions were also analyzed to determine whether any enamel dissolved during treatment, but no evidence of dissolved enamel was found in any of these solutions. A comparison of these compounds at identical pH values is not included in this presentation, as such changes in pH completely alter the nature of the materials and do not necessarily make any one of the materials more comparable to the others. For example, acidic sodium fluoride, which contains substantial amounts of HF, causes severe damage to enamel, and stannous ions precipitate completely from neutral stannous solutions as the oxide salt.[11]

Results of the analyses for calcium and phosphate were calculated to weight of enamel using a published composition[9] which had also been checked by analyses of known weights of enamel. The weight of enamel was expressed as mg. of enamel dissolved per cm.^{2} of enamel surface exposed, to provide a common basis for comparison of all the different experiments. The surface area was measured from photographic enlargements of the enamel samples. The coefficient of variation for the measurement of the dissolution rate was ±6 per cent among enamel samples, with a maximum range of ±10 per cent. Each point shown on the graphs represents a single measurement, except for the control curve for untreated enamel by the differential method. This curve is an average of 31 runs on 16 different enamel samples. The differential curves were also checked with replicate experiments and were all within the variation cited above.

The acid solution used throughout these experiments was 0.1 M lactic acid* adjusted to pH† 4.5 with NaOH. Lactate polymers were decomposed by aging stock solutions for several months, and acid strength was checked by titration with NaOH. Stannous fluoride‡ (0.1 per cent, pH 3.4) and stannous chloride‡ (0.484 per cent, pH 2.2) solutions were prepared by weight giving 3,030 ppm Sn(II) and 970 ppm F$^-$ and were checked by an iodine titration for Sn(II). Sodium fluoride (0.2 per cent) was prepared by weight giving 905 ppm F$^-$, and pH was adjusted to 7.0 as necessary with either HCl or NaOH.

Results

SOUND ENAMEL. — The dissolution rate for sound enamel previously treated for 5 minutes with SnF_2, $SnCl_2$, or NaF is presented in Figures 2, 3, and 4. Using the differential method (Fig. 2), essentially the same reduction in solubility rate was obtained for all three materials. The primary effect of the treatment occurred during the first few minutes or in the first two acid exposures. Thereafter, the slopes of these curves became parallel with the curve for untreated enamel, demonstrating that the rate had returned to that of normal sound enamel. Obviously, the effect of all the treatments was confined primarily to the surface layers of enamel. This result is consistent

* Reagent Grade, J. T. Baker Chemical Co., Phillipsburg, N.J.

† Beckman Model G pH Meter, Beckman Instruments, Inc., Fullerton, Calif.

‡ M. & T. Corporation, Rahway, N.J.

with Cooley's findings[9] of slight penetration of fluoride and Sn(II) into sound enamel.

The results by the integral method are shown in Figures 3 and 4. Figure 3 is drawn to the same scale as Figure 2 for comparison purposes, while the greater extent of the curve is given in Figure 4. Treatment with

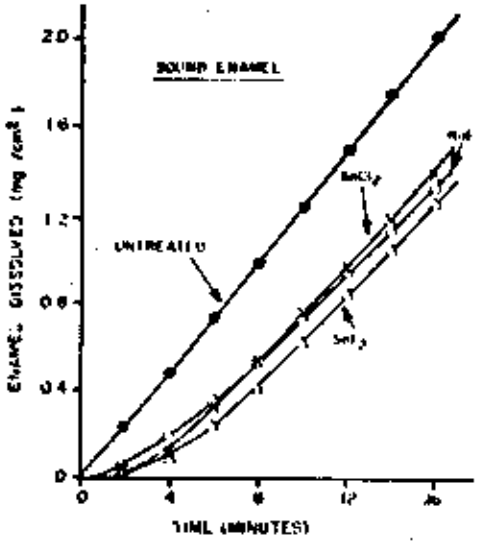

FIG. 2. The inhibiting effect of treatment with SnF_2, $SnCl_2$, or NaF on the subsequent enamel dissolution rate in acidic buffer using sound enamel and a differential method. Five minute treatment, 3,000 ppm Sn(II), 950 ppm F^-, 0.1 M pH 1.5 lactate buffer, 37° C.

NaF caused a response in enamel solubility reduction typical of that found by other investigators.[1-8] The dissolution rate remained less than for normal enamel at all times studied. Treatment with SnF_2 was more effective than the NaF treatment in reducing the rate for the first hour or so but then came to a final value comparable to that found for treatment with NaF. This result suggests that the effect of Sn(II) had been depleted and only a residual effect of fluoride remained. The $SnCl_2$ treatment initially had a very great effect, matching that of SnF_2, but within a 1-hour period the effect was lost completely, and the dissolution rate became equivalent to the no-treatment case.

ENAMEL WITH SUBSURFACE DECALCIFICATION.—The dissolution rates for subsurface decalcified enamel treated in a fashion similar to the normal sound enamel are presented in Figures 5-7. The rate for untreated enamel with subsurface decalcification (after polishing) was the same as the normal sound enamel in the case of the differential method, and, therefore, the results of the normal enamel were used as the control curve. Using the differential method (Fig. 5), results were very different in this respect from those obtained with sound enamel. The NaF or $SnCl_2$ treatment reduced the solubility a modest amount and about to the same extent as found with sound enamel. The SnF_2 treatment, however, was very effective, much more so than

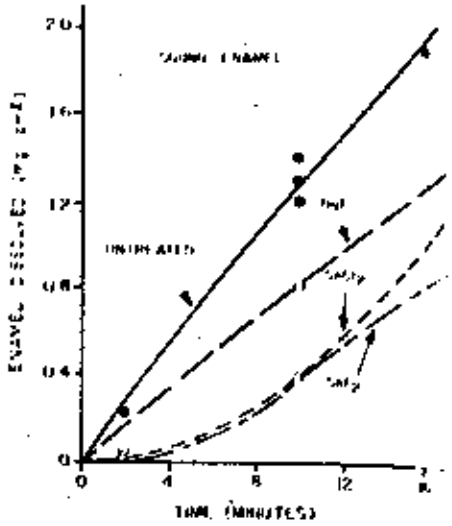

FIG. 3. The inhibiting effect of treatment with SnF_2, $SnCl_2$, or NaF on the subsequent enamel dissolution rate in acidic buffer using an integral method. Only the initial portions of the curves (from Fig. 1) are shown for comparison to the same scale with Fig. 2. Five minute treatment, 3,000 ppm Sn(II), 950 ppm F^-, 0.1 M pH 1.5 lactate buffer, 37° C.

found on sound enamel (cf. with Fig. 4) causing a startling difference between NaF and either NaF or $SnCl_2$. The measurements by the integral method (Figs. 6 and 7) were consistent with the pattern shown by the results of the differential method. NaF treatment reduced the solubility rate for all exposure times studied and to an extent comparable to that found for sound enamel (cf. Fig. 7 with Fig. 1). Again, $SnCl_2$ treatment was more effective than NaF treatment

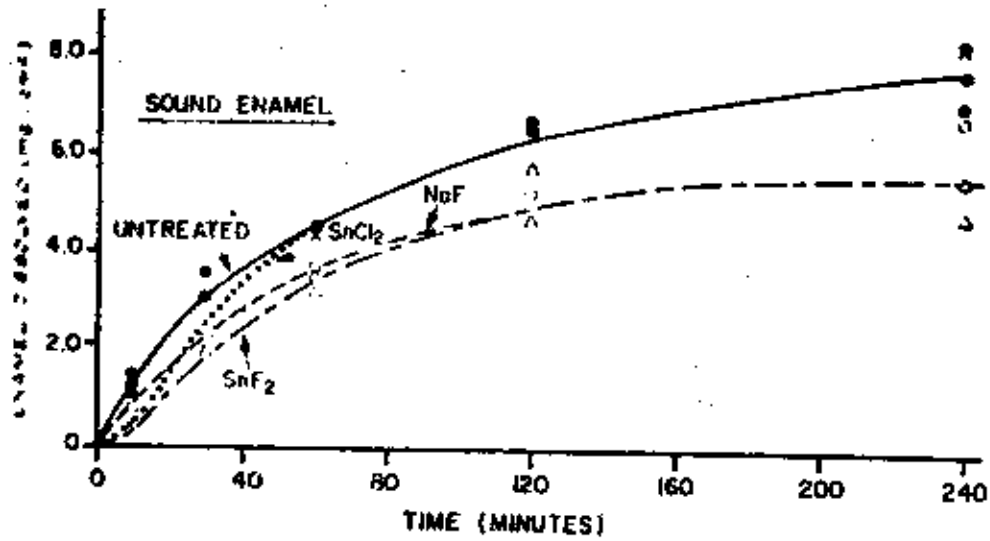

Fig. 4. Using an integral method, the inhibition of sound enamel dissolution rate in 0.1 M, pH 4.5 lactate buffer at 37°C resulting from a 5-minute treatment with SnF_2, $SnCl_2$, or NaF; 3,000 ppm Sn(II), 950 ppm F⁻.

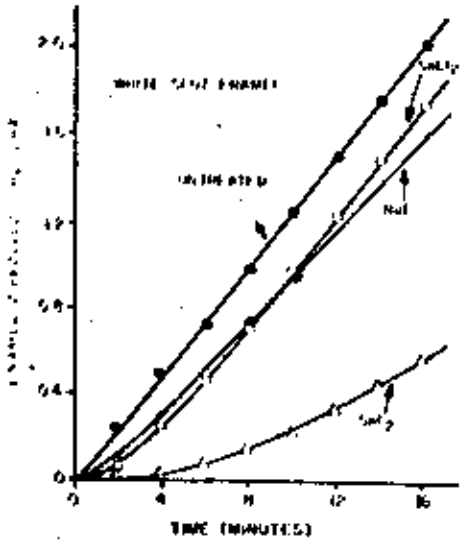

Fig. 5. Using decalcified enamel (i.e., with a "white spot" surface) and a differential method, the inhibition of the dissolution rate in acidic buffer resulting from a treatment with SnF_2, $SnCl_2$, or NaF. Five-minute treatment, 3,000 ppm Sn(II), 950 ppm F⁻, 0.1 M pH 4.5 lactate buffer, 37°C.

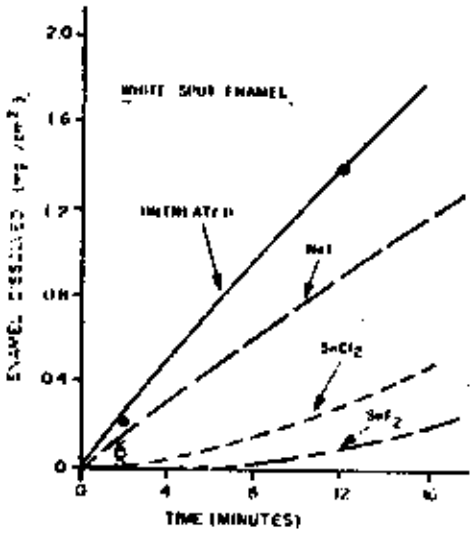

Fig. 6. Using an integral method and decalcified enamel, the inhibition of the dissolution rate in acidic buffer resulting from a treatment with SnF_2, $SnCl_2$, or NaF. The initial portions of the curves of Fig. 7 are drawn here to the same scale as Figs. 2, 3, and 5 for comparison purposes. Five-minute treatment, 3,000 ppm Sn(II), 950 ppm F⁻, 0.1 M pH 4.5 lactate buffer, 37°C.

initially, but eventually the protection of $SnCl_2$ was lost completely, and the rate became equivalent to that of untreated enamel. Treatment with SnF_2 was extremely effective in reducing the dissolution of enamel for short exposure times and maintained, throughout the exposure period examined, a high degree of efficacy, which was always greater than that afforded by treatment with NaF.

It was mentioned previously that the enamel surface was lightly polished after the exposure to the decalcification medium used to produce the incipient caries-like lesion. This was done because the original surface of the incipient caries-like lesion was less soluble (and the solubility less reproducible) than polished sound enamel, a result similar to what has been found for natural incipient carious lesions.[26] The result of not polishing, but only wiping the surface with a paper tissue, is presented in Figure 8. The wiped sample was less soluble than normal sound enamel (in agreement with Muhlemann[7]) or the polished sample of enamel with a lesion, while the latter two were equivalent. The polishing did not remove the lesion, which was still visible to the eye, and gave a response to treatment with SnF_2 different from sound enamel, as discussed in the previous paragraph. The unpolished surface also responded differently to the treatment with SnF_2 and $SnCl_2$. The sodium fluoride treatment gave the same solubility [illegible] (Fig. 8) regardless of the surface condition, suggesting that, in this case, only the surface conditioning effect was being measured. Treatment with either SnF_2 or $SnCl_2$ [illegible] much greater decreases in the solubility [illegible] than was found on polished surfaces, suggesting a greater uptake and affinity of [illegible] and $SnCl_2$ for the unpolished surface. The presence of an organic deposit formed during exposure to the decalcification medium, corresponding to the acquired enamel [illegible], would make the increased reactivity to $SnCl_2$ very plausible.

Discussion

The different shapes of the solubility curves depending on time, type of treatment, and condition of enamel can lead to widely varying results in different [illegible] studies incorporating single sets of exposure times. For example, it is possible to compare the effect of choosing different acid exposure times from Figures 1 and 7 for determination of the per cent solubility reduction (Table 1). For a very short acid exposure time (e.g., 10 minutes), treatment with SnF_2 and $SnCl_2$ would be about equivalent and even better than treatment with NaF, [illegible] either sound or decalcified enamel. If [illegible]

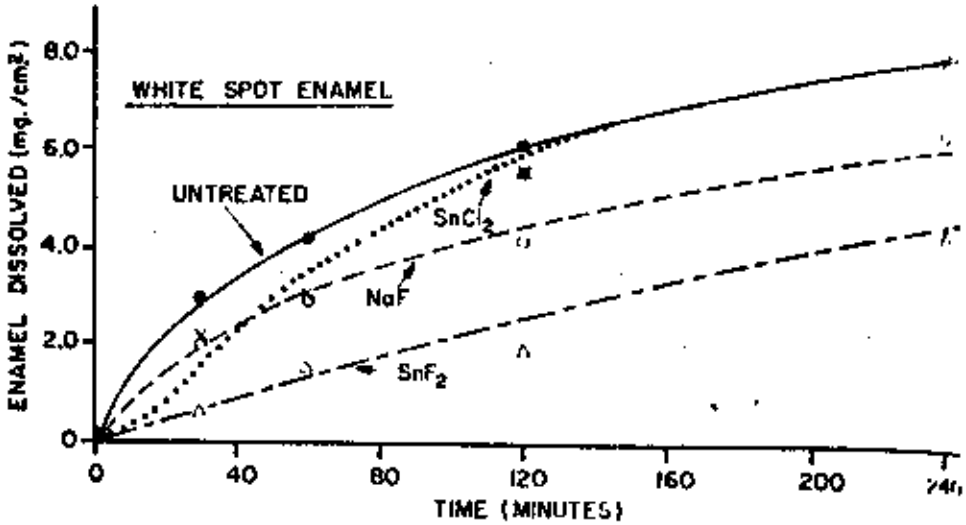

FIG. 7. The inhibiting effect of treatment with SnF_2, $SnCl_2$ or NaF on the subsequent dissolution rate of decalcified enamel using an integral method. Five minute treatment, 3,000 ppm SnF_2, 9,400 ppm F, 0.1 M pH 4.5 lactate buffer, 37° C.

TABLE 1

PER CENT ENAMEL SOLUBILITY* REDUCTION FOR DIFFERENT ACID-EXPOSURE TIMES AND ENAMEL CONDITIONS

Treating Agent†	10 Minutes		30 Minutes		120 Minutes	
	Sound Enamel	White-Spot Enamel	Sound Enamel	White-Spot Enamel	Sound Enamel	White-Spot Enamel
SnF_2	56	87	34	78	23	52
$SnCl_2$	44	67	14	34	0	6
NaF	33	33	24	28	23	28

* 0.1 M, pH 4.5 lactate buffer, 37° C.

† 5-minute treatment, 3,000 ppm Sn(II), 950 ppm F^-.

minute acid-exposure test period were selected, $SnCl_2$ and NaF treatment would become equivalent in efficacy, while SnF_2 treatment would remain better, and much more so by using decalcified enamel. Finally, if a long time-interval were chosen, NaF treatment would be better than $SnCl_2$ treatment (which would now be completely ineffective), and SnF_2 treatment would be equivalent to NaF treatment on sound enamel and twice as effective on decalcified enamel. Thus a wide variety of results could be obtained by simply altering the length of the acid exposure. However, regardless of the technique, treatment with SnF_2 is always as good as or better than treatment with either of the other two materials.

The absolute or quantitative positions of the curves could be shifted by increasing treatment time, treatment concentration, acid strength, or enamel sample size; but the relationship of one curve to the other should remain about the same.

In human clinical studies using higher

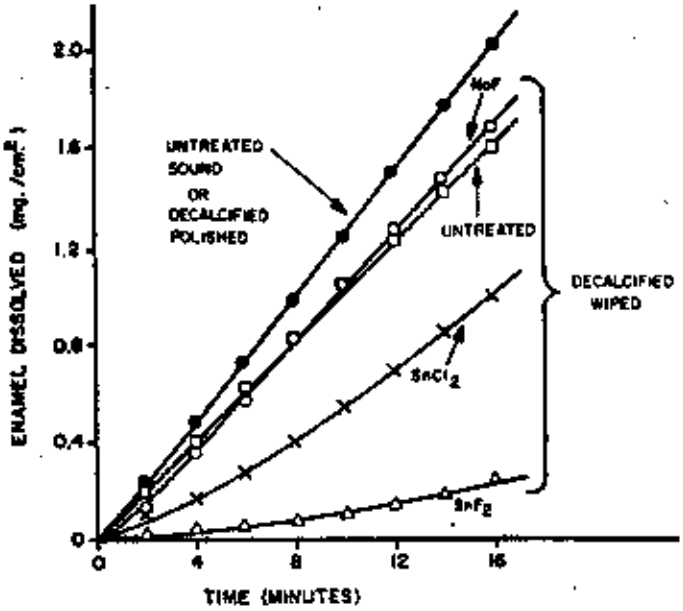

FIG. 8.—The effect of wiping instead of polishing the white-spot enamel after producing the subsurface calcification in vitro. The unpolished decalcified enamel was less soluble, and its solubility rate in acidic buffer was inhibited more by treatment with SnF_2 or $SnCl_2$ (cf. Fig. 5). Five-minute treatment, 3,000 ppm Sn(II), 950 ppm F^-, 0.1 M pH 4.5 lactate buffer, 37° C.

concentrations and annual topical applications,[27] it has been shown that treatment with stannous fluoride reduced dental caries more than did treatment with sodium fluoride, although both agents were effective. A non-fluoride stannous salt was found to have no effect.[28] Similar results have also been obtained in animal studies.[29, 30] The set of conditions in the solubility studies yielding these same results (i.e., $SnF_2 > NaF > SnCl_2$ = no effect) was the use of enamel with an incipient caries-like lesion and a long acid exposure. The same result can be achieved with shorter exposure times by increasing the acid strength. The results obtained by differential and integral methods lead to the same conclusions, but the integral method is more sensitive to small differences, while the differential method separates the highly effective materials and permits more understanding of the mechanism.

The results certainly point out the hazards of determining the in vivo efficacy of a material from its effect on the solubility of enamel in acid without a rather complete comprehension of the action of the material on enamel dissolution rate and some cross-checks with performance in humans. In this connection the use of enamel with an incipient caries-like lesion gave a solubility performance more closely aligned with clinical performance than did the use of sound enamel. There are other good reasons for examining the effect of anticariogenic agents on the solubility of enamel which has an incipient caries-like lesion. First, it is logical to examine the damaged tissue, the object of the search for preventive or reparative measures, as well as to study the sound, healthy material. Second, results of clinical investigations have led to speculation that SnF_2 may be exerting a great effect on this early stage of caries. Finally, it has been shown that these defects take up large amounts of Sn(II) and fluoride,[31] and this greater reservoir of antisolubility agent should then exert a more marked or prolonged effect. The rapid hydrolysis of $SnCl_2$ above pH 3 prevents the penetration of Sn(II) into these defects, and only the surface effect is found, just as with sound enamel. However, sound enamel is still the best substrate for study of the reaction, reaction products, and changes in physical properties of enamel and can be used to good advantage in combination with any other type of enamel solubility study.

Summary

Dental enamel, both sound and with incipient caries-like lesions, was treated with SnF_2, $SnCl_2$, or NaF at 3,000 ppm Sn(II) and 950 ppm F^-. The dissolution rate of the treated enamel was measured in lactate buffer by both a differential and an integral method. Treatment with stannous fluoride reduced the dissolution rate of either sound or white-spot enamel, and the effect of this treatment was always equal to or greater than that effected by treatment with $SnCl_2$ or NaF under comparable conditions. Sodium fluoride treatment also reduced the enamel dissolution rate under all conditions but $SnCl_2$ treatment caused an inhibition of limited duration that was eventually lost completely. Changing the acid-exposure time altered the relationship of enamel dissolution rate effects produced by the various agents, and this could lead to contradictory conclusions from solubility studies using different dissolution conditions. The results of using enamel with incipient caries-like lesions correlated more closely with human clinical investigations than did the results from sound enamel.

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Acute and Chronic Heavy Metal Poisoning.

II. Effects of Divalent Tin.

by

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In the present work the effects of an aromatic complex salt of tin with a pyrocatechol derivative (Heyden 768[1]), and of an aliphatic complex salt, stannous potassium tartrate (divalent Sn content 32%) were studied. According to our analyses (see below) the aromatic complex salt contained 9.5% divalent tin. In acute toxicity experiments both complex salts gave rise to symptoms of heavy metal poisoning. Signs of paralysis developed within a short time, starting in the hind legs, then rising until the animals died in an asphyxial seizure. 0.15 g Sn/kg, with subcutaneous application of the aromatic complex salt, proved to be the acutely lethal dose for mice, guinea pigs and rabbits. The aliphatic complex salt had a lethal effect on these animals in a concentration of 0.07 g Sn/kg.

Upon chronic application the aromatic preparation proved to have a very low toxicity. Large doses were required to kill the animals: e.g. 184 mg/kg rabbit in 14 days. With 90 mg Sn/kg/week two animals were killed in two weeks; with 80 mg/kg/week one animal died after 3 1/2 weeks, with 70 mg/kg/

week one animal died after 6 1/2 weeks. Here again we were struck by the basic difference between acute and chronic heavy metal poisoning. In chronic experiments the aliphatic preparation also proved to be considerably more toxic. 50 mg Sn/kg rabbit was lethal within 4-8 days. Table 1 gives details of the experiments (see also rabbit 11, table 3). It should be recalled that the manganous compound prepared with the same complex component proved considerably less toxic in chronic experiments than manganous citrate[2].

Table 1.

Tabelle 1.

1 Kaninchen 67, ♀, 2000 g Gewicht, erhält pro Kilogramm und Injektion 0,023 g Sn (aromatisches Komplexsalz).

Injektion am	2 Bemerkungen
11. V.	—
13. V.	—
15. V.	—
16. V.	3 —
18. V.	2000 g Gewicht.
20. V.	4 —
22. V.	Apathisch, frißt wenig.
25. V.	5 " " "
	Am 2. VI. †, 1850 g Gewicht.

6 Im ganzen 0,368 g Sn = 0,184 kg in 14 Tagen.
7 Sektion: fettige Degeneration der Leber, sonst ohne Befund.

8 Kaninchen 66, ♀, 2250 g Gewicht, erhält pro Kilogramm und Injektion 0,023 g Sn (aliphatisches Komplexsalz).

Injektion am	9 Bemerkungen
11. V.	10 Matt, frißt wenig.
15. V.	11 —
	Am 17. V. †.

11 Im ganzen 0,046 g Sn/kg in 4 Tagen.

1. Rabbit 67, weight 2000 g, female, received 0.023 g Sn (aromatic complex salt) per kg and injection. 2. Remarks. 3. Weight 2000 g. 4. Apathetic, poor appetite. 5. Died on June 2, weight 18500 g. 6. Total 0.368 g Sn = 0.184 kg in 14 days. 7. Autopsy: fatty degeneration of the liver, otherwise normal. 8. Rabbit 66, female, weight 2250 g, received 0.023 g Sn (aliphatic complex salt) per kg and injection. 9. Remarks. 10. Dull, poor appetite. 11. Died May 17. 11. Total 0.046 g Sn/kg in 4 days.

In the chronic toxicity experiments with the aromatic compound we were struck by a marked increase in urine quantity. To establish the cause, the urinary and fecal excretions of the animals were examined for tin. We did not analyze the tin content of the individual organs, since the sites of highest concentration are not the sites of severest disturbance, so that the distribution of the poison in individual organs has no direct connection with the symptoms of poisoning.

First a brief description will be given of the method of tin determination, which we, together with Dr. Schulz, have found to be the best after prolonged preliminary experiments.

Dissolution of the fecal and urinary samples in sulfuric acid (only 5 cc at the most) and nitric acid was the same as for manganese determination. Here again the entire urine quantity was analyzed, but only 10 g of the fecal samples (see earlier ref.)

Ashing with nitric acid must be pursued until complete colorlessness of the solution is achieved, which often took a very long time and for which a great deal of nitric acid was necessary.

A salt-like residue precipitated in large quantities, particularly in the case of the urine samples; this was found to be partly insoluble, even upon later dilution with water.

After complete ashing all the nitric acid still present was removed. The solution was heated until ample sulfuric acid vapor developed, after cooling it was diluted to c. 100 cc with water and boiled once more for a certain period, so as to remove any nitrosyl sulfuric acid and all nitrogen oxides that may have been produced.

No tin precipitates, for instance, in the form of ~~stannic~~ tin oxide upon ashing. All the tin is contained in the sulfuric acid solution. Clear tin solutions, the tin content of which was in accord with the samples under study, were treated in exactly the same way as these with sulfuric and nitric acid. The similarly diluted sulfuric acid solution was clear, nothing had precipitated.

The diluted sulfuric acid solution obtained in this manner was filtered off from the insoluble residues and the residue washed with warm, weakly sulfuric acid water. Hydrogen sulfide was introduced into this solution in a closed Erlenmeyer and it was once again diluted with a small amount of water. In view of the small quantities of tin present, only a few milligrams, as a rule there is no precipitation upon introducing hydrogen sulfide. The solution then assumed a more or less yellow-brown color. Next it was heated for a certain length of time in the water bath until the odor of hydrogen sulfide had disappeared. The tin precipitated as ~~stannic~~ Tin sulfide in the well-know yellow-brown form. The precipitate

was then filtered off and washed with water.

The sulfide was converted into stannic dioxide by ashing and weighed as such. Small porcellain crucibles were used, which were very easily kept constant to fractions of a tenth of a milligram. Weighing took place on a Sartorius micro-balance. Great stress was laid on temperature adjustment. The filtered ash from the two small filters (diameter c. 3 cm) weighed c. 0.01-0.02 mg. The quantity was thus so small that it could be disregarded.

Table 2 shows the diuretic effect of the aromatic and aliphatic tin compounds on the rabbit.

Table 2.

Tabelle 2.

1 Kaninchen 64, ♀, 1850 g Gewicht: Aromatisches Komplexsalz.

2 Datum	3 Gewicht in g	4 Harnmenge in ccm	5 Injektion 23 mg Sn/kg am
17. IV.—24. IV.	1850	245	—
24. IV.— 1. V.		280	—
1. V. — 8. V.		340	—
8. V. —15. V.		630	8. V.
			11. V.
			14. V.
15. V. —21. V.	1945	820	16. V.
			19. V.
			22. V.
22. V. —29. V.		1220	—
29. V. — 5. VI.		1250	—
26. VI.— 3. VII.	2130	980	—
1. IX. — 8. IX.		580	—

6 Kaninchen 65, ♀, 2250 g Gewicht: Aromatisches Komplexsalz.

Datum	Gewicht in g	Harnmenge in ccm	Injektion 23 mg Sn/kg am
17. IV.—24. V.	2250	280	—
24. IV.— 1. V.		260	—
1. V. — 8. V.		290	—
8. V —15. V.		500	8. V.
			11. V.
			14. V.
15. V. —22. V.	2225	615	16. V.
			19. V.
			22. V.
22. V. —29. V.		865	—
29. V. — 5. VI.		860	—
26. VI.— 3. VII.	2250	740	—
1. IX.— 8. IX.		300	—

7 Kaninchen 18, ♀, 2300 g Gewicht: Aliphatisches Komplexsalz.

Datum	Harnmenge in ccm	Injektion 4,5 mg Sn/kg am	8 Bemerkungen
11. VI. —18. VI.	380	—	—
18. VI. — 25. VI.	400	—	9 —
25. VI. — 2. VII.	315	25. VI.	29. VI.: Apathisch, frißt kaum.
		27. VI.	—
		28. VI.	—
2. VII. — 9. VII.	220	2. VII.	10 —
9. VII.—12. VII.	60	—	12. VII.: Tot.

1. Rabbit 64, female, 1850 g, aromatic complex salt. 2. Date. 3. Weight in g. 4. Urine quantity cc. 5. Injection, 23 mg Sn/kg on: Date. 6. Rabbit 65, female, 2250 g, aromatic complext salt. 7. Rabbit 18, female 2300 g, aliphatic complex salt. 8. Remarks. 9. June 29: apathetic, scarcely eats. 10. July 12, dead.

Table 3 gives an abstract of the experimental protocols for the uptake and excretion of tin (as aromatic compounds) in two rabbits.

Tabelle 3.

1 Aromatischen Komplexsalz.

Kaninchen 11, ♀, 1920 g Gewicht					Kaninchen 12, ♀, 2000 g Gewicht			
2 3 Harn		4 Kot		5 Datum	Harn		Kot	
6 Menge in ccm	Sn in mg	7 Menge in g	Sn in mg		Menge in ccm	Sn in mg	Menge in g	Sn in mg
575	5,70	89	10,33	23. II. — 2. III.	600	7,21	62	7,57
1175	6,24	132	18,00	2. III — 9. III.	750	5,13	34	5,92
1240	3,76	51	8,63	9. III.—16. III.	660	2,48	95	5,16
1210	5,05	100	15,20	16. III —23. III.	900	4,98	116	10,14
900	6,00	—	30,21	23. III —30. III.	850	8,22	—	12,28
450	7,11	170	75,19	30. III — 6. IV.	980	12,14	105	16,63
880	3,60	172	25,06	6. IV.—14. IV.	830	16,42	—	20,31
				14. IV.—17. IV.	530	1,41	36	24,07

8 Kaninchen 11 erhält Injektion: Am 23. II. 34 mg Sn/kg; 26. II., 1. III., 5. III., 8. III., 12. III., 15. III., 17. III., 20. III., 23. III., 25. III., 27. III., 30. III., 1. IV., 3. IV., 5. IV. je 23 mg Sn/kg; 6. IV. 40 mg Sn/kg. † 14. IV.

9 Sektion: Leber: Fettige Degeneration: in den übrigen Organen, auch in den Nieren makroskopisch und mikroskopisch kein pathologischer Befund. Wochenharnmenge normal: 290 ccm.

10 Kaninchen 12 erhält Injektion: Am 23. II. 34 mg Sn/kg; 26. II., 1. III., 5. III., 8. III., 12. III je 23 mg Sn/kg. Wochenharnmenge normal: 320 ccm.

11 Kaninchen 11

12 erhält injiziert . . .	813	mg Sn	23. II.—6. IV.
13 scheidet aus im Kot.	181,90	» »	
14 » » » Harn	37,40	» »	
15 insgesamt	219,30	mg Sn	

16 Kaninchen 12

12 erhält injiziert . . .	257	mg Sn	23. II. bis 16. III.	0,00 mg Sn	16. III. bis 13. IV.
scheidet aus im Kot.	18,65	» »		83,43 » »	
14 » » Harn	14,80	» »		43,17 » »	
15 insgesamt	33,45	mg Sn		126,60 mg Sn	

1. Aromatic complex salt. 2. Rabbit 11, female, 1920 g. 3. Urine. 4. Feces. 5.Date. 6. Quantity cc. 7.Quantity in g.8. Rabbit 11 received injections on: Dates and quantities of Sn. Died Apr.14. 9. Autopsy: Liver: Fatty degeneration. No pathological findings, either macroscopic or microscopic in other organs, including kidneys. Normal weekly urine quantity: 290 cc. 10. Rabbit 12 received injections on: date and Sn quantities. Weekly urine quantity normal: 320 cc. 11. Rabbit 11. 12. Received in injection: 13. Excreted in feces: 14. Excreted in urine. 15. Total.

It is apparent from these experiments that considerably more tin is excreted in the feces than in the urine; as in the case of other heavy metals, the unusually slow excretion is striking in this case also. Four weeks after cessation of the tin injections considerable quantities were still being excreted in the urine and feces. The unusually long duration of increased diuresis is probably linked to this slow excretion. The time of onset of diuresis varies: in 8 cases after the second injection, 3 times after the third, 3 times after the fourth injection of 23 mg Sn/kg.

A diuretic effect could also be elicited in dogs treated with the aromatic tin compound (see table 4).

1. Dog, male, 6000 g. 2. Date. 3. Urine quantity cc. 4. Av. day urine quantity cc.

Tabelle 4.

1 Hund, ♂, 6000 g Gewicht.

2 Datum	Injektion 20 mg Sn/kg	3 Harnmenge in ccm	4 Durchschnittliche tägliche Harnmenge in ccm
7. XI.—16. XI.	—	1445	161
16. XI.—25. XI.	16. XI.	2005	223
	19. XI.	—	—
	23. XI.	—	—
25. XI.—30. XI.	—	1680	336
30. XI.— 9. XII.	—	1395	155

Local reactions were not observed upon injection of the aromatic complex salt. The dogs suffered pain from the injection and in humans (self-experiment) the injection of 1 cc solution containing 1% Sn was not extremely, but nevertheless definitely painful.

The diuretic effect of the complex component was of course also investigated, but increased diuresis was never observed. We did not observe a diuretic effect with the aliphatic complex compound either (see Table 2), but of course we could not use anything approaching the high concentrations of the aromatic compound.

The kidneys of the animals were examined under the microscope both for the degree of diuresis (4 animals) and its attenuation (4 animals), since there was a suspicion of inflammatory irritation. However, the kidneys in all cases revealed no pathological findings (8 animals were examined); no protein was ever found in the urine.

The causes of the diuretic effect of tin in an appropriate combination and concentration could not be determined more closely any more than those of mercury. In acute experiments, as was predictable, no diuretic effect could be achieved with intravenous injections (catheterization of ureters, perfusion of a solution containing 1% Sn into the ear vein, 13.5 cc in 24 min, 16 cc in 15 min, 25 cc in 16 min, no diuretic effect).

The peculiar distribution of the excreted tin in the urine and feces led us to study the mode of excretion of other heavy metals in the literature, so far as feasible. It is known concerning Ca that it can be made to predominate in urinary or fecal excretion according to the phosphate content and the H-ions in the blood. Table 5 shows the mode of excretion of heavy metals.

Table 5.

Tabelle 5.

1. Metall	2. Atomgewicht	3. Ausscheidung Kot — Harn	Literatur 6
Al	27	K ≧ H	Kunkel, Toxikologie.
Mn	55	0	Handovsky-Schulz-Stammler, a.a.O.
Fe	56	K ≧ H	7 Vgl. Kunkel, a.a.O., S.176; ferner Kobert, Dorpater Arbeiten VII.
Ni	59	K ≧ H	Kunkel, a.a.O., S. 178; Kobert, Toxikologie, S 59.
Zn	65	K > H	Kobert, Dorpater Arbeiten IX.
Cu	63	K ≧ H	Kobert, a.a.O.
Ag	108	K > H	Kobert, Dorpater Arbeiten IX.
Sn	119	75:25	Handovsky.
As	197	K < H	Heubner, Dieses Archiv, Bd. 58.
Hg	201	30:70	Lomholt, Biochem. Journ. 1924. Bd. 18. S. 69.
Pb	207	45:55	
Bi	208	35:65	

1. Metal. 2. Atomic weight. 3. Excretion. 4. Feces. 5. Urine. 6. as cited earlier. 7. See Kunkel, as cited earlier, p. 176; also Kobert, Dorpater Works, VII.

The data in Kobert's and Kunkel's works on Toxicology are not directly comparable with those of Lomholt and our own, since the former were not dealing with chronic poisoning of rabbits. For this reason we have only cited those metals concerning which the authors have definitely pointed out that appreciably more is excreted in the feces or urine upon subcutaneous administration.

We believe, however, that it may be concluded from this table that heavy metals with a low atomic weight are excreted predominantly in the feces, while those with a high atomic weight predominantly in the urine. Which qualities linked to the atomic structure (oxide formation, position according to charge) are responsible for this unusual biological behavior of heavy metals is not known. It is conceivable that it is

necessary for a diuretic effect that an appreciable proportion of the metal be excreted through the kidneys. In tin we have a metal that is excreted in sufficient quantities through the kidneys, but in contrast to mercury, does not cause nephritis. Thus we have an effective and yet harmless metal diuretic, although fairly large quantities are required to elicit diuresis.

Footnotes.

1. The constitution will be published in a chemical journal by the author who prepared it.

2. This journal, 1925, Vol. 110, p. 265.

3. Small losses.

VI.

Aus dem Pharmakologischen Institut der Universität Göttingen.

Über akute und chronische Schwermetallvergiftungen.

II. Mitteilung: Wirkungen des zweiwertigen Zinns.

Von

Hans Handovsky.

(Eingegangen am 10. IV. 1928.)

In der vorliegenden Untersuchung wurden die Wirkungen eines aromatischen Komplexsalzes des Zinns mit einem Brenzkatechinderivat (Heyden 768) [1]) und eines aliphatischen Komplexsalzes, des weinsauren Zinnkaliums (Gehalt an 2wertigem Sn 32%) untersucht. Das aromatische Komplexsalz enthielt nach unseren Analysen (s. unten) 9,5% 2wertiges Zinn; es wurde stets eine frisch hergestellte 9,5%ige Lösung verwandt, die 1% Zinn enthielt. In akuten Vergiftungsversuchen zeigten beide Komplexsalze die Erscheinungen der Schwermetallvergiftung, es traten in kurzer Zeit Lähmungserscheinungen auf, die an den hinteren Extremitäten begannen, dann aufstiegen, bis die Tiere unter asphyktischen Krämpfen zugrunde gingen. Als akut tödliche Dosis für Mäuse, Meerschweinchen und Kaninchen erwiesen sich 0,15 g Sn/kg bei subkutaner Applikation des aromatischen Komplexsalzes; das aliphatische Komplexsalz wirkte auf diese Tiere in einer Konzentration von 0,07 g Sn/kg tödlich.

Bei chronischer Applikation erwies sich das aromatische Präparat als sehr wenig giftig, man brauchte große Dosen, um die Tiere zu töten: z. B. 184 mg/kg Kaninchen in 14 Tagen. Bei 90 mg Sn/kg und Woche gelang es, zwei Tiere in 2 Wochen zu töten; bei 80 mg/kg und Woche starb ein Tier nach 3½ Wochen; bei 70 mg/kg und Woche starb ein Tier nach 6½ Wochen. Es fällt uns auch hier

1) Die Konstitution will der Darsteller selbst in einer chemischen Zeitschrift veröffentlichen.

der prinzipielle Unterschied zwischen der akuten und chronischen Schwermetallvergiftung auf. Auch in den chronischen Versuchen erwies sich das aliphatische Präparat als wesentlich giftiger. 50 mg Sn/kg Kaninchen töteten innerhalb 4—8 Tagen. Über die Versuche gibt die folgende Tabelle 1 Aufschluß (vgl. auch Kaninchen 11, Tabelle 3). Es sei daran erinnert, daß auch die mit demselben Komplexbildner hergestellte Manganoverbindung sich im chronischen Versuch als wesentlich weniger giftig erwiesen hat als Manganocitrat[1]).

Tabelle 1.

Kaninchen 67, ♀, 2000 g Gewicht, erhält pro Kilogramm und Injektion 0,023 g Sn (aromatisches Komplexsalz).

Injektion am	Bemerkungen
11. V.	—
12. V.	—
14. V.	—
15. V.	—
19. V.	2000 g Gewicht.
20. V.	—
22. V.	Apathisch, frißt wenig.
23. V.	» » »
	Am 2. VI. †, 1850 g Gewicht.

Im ganzen 0,368 g Sn = 0,184 kg in 14 Tagen.
Sektion: fettige Degeneration der Leber, sonst ohne Befund.

Kaninchen 66, ♀, 2250 g Gewicht, erhält pro Kilogramm und Injektion 0,023 g Sn (aliphatisches Komplexsalz).

Injektion am	Bemerkungen
11. V.	Matt, frißt wenig.
13. V.	—
	Am 17. V. †.

Im ganzen 0,046 g Sn/kg in 4 Tagen.

Sehr auffällig war bei den chronischen Vergiftungsversuchen mit der aromatischen Verbindung eine starke Zunahme der Harnmenge. Um sie aufzuklären, haben wir die Ausscheidungen der Tiere im Kot und Harn auf Zinn analysiert. Eine Analyse der einzelnen Organe auf ihren Zinngehalt haben wir nicht vorgenommen, da ja die Stätten höchster Konzentration nicht die Stätten der größten Störungen sind,

[1]) Dieses Archiv 1925, Bd. 110, S. 265.

somit die Verteilung des Giftes in den einzelnen Organen keinen unmittelbaren Zusammenhang mit den Symptomen der Vergiftung hat.

Zunächst sei die Methode des Zinnnachweises kurz beschrieben, die sich uns gemeinsam mit Herrn Dr. Schulz nach längeren Vorversuchen als die beste erwiesen hat.

Das Lösen der Kot- und Harnproben in Schwefelsäure (meistens nur 5 ccm) und Salpetersäure erfolgte wie bei der Manganbestimmung. Auch hier wurden die gesamten Harnmengen verarbeitet, von den Kotproben nur je 10 g (vgl. a. a. O.).

Die Veraschung mit Salpetersäure hat hier bis zur vollständigen Farblosigkeit der Lösung zu erfolgen, was oft sehr lange dauerte, und wozu viel Salpetersäure benötigt wurde.

Besonders bei den Harnproben schied sich in großer Menge ein salzartiger Rückstand ab, der auch bei dem späteren Verdünnen mit Wasser nur zum Teil löslich war.

Nach vollständiger Veraschung wurde sämtliche noch vorhandene Salpetersäure entfernt. Die Lösung wurde bis zur starken Entwicklung von Schwefelsäuredämpfen erhitzt, nach dem Abkühlen mit Wasser auf etwa 100 ccm verdünnt und nochmals einige Zeit gekocht, um etwa entstandene Nitrosylschwefelsäure und sämtliche Stickstoffoxyde zu entfernen.

Bei der Veraschung scheidet sich kein Zinn etwa als Zinnoxyd aus. Alles Zinn ist in der schwefelsauren Lösung enthalten. Klare Zinnlösungen, deren Gehalt an Zinn den zu untersuchenden Proben entsprach, wurden genau so mit Schwefel- und Salpetersäure behandelt wie diese. Die ebenso verdünnte schwefelsaure Lösung war klar, es hatte sich nichts ausgeschieden.

Die so erhaltene verdünnte, schwefelsaure Lösung wird von den unlöslichen Rückständen abfiltriert, und der Rückstand mit warmem, schwach schwefelsaurem Wasser ausgewaschen.

In diese Lösung wird in einem verschlossenen Erlenmeyer Schwefelwasserstoff eingeleitet, wobei nochmals mit etwas Wasser verdünnt wird. Bei den geringen Zinnmengen, die vorhanden sind, nur einigen Milligrammen, fällt beim Einleiten gewöhnlich kein Niederschlag aus. Die Lösung färbt sich nur mehr oder weniger gelbbraun. Sie wird dann auf dem Wasserbad längere Zeit erwärmt, bis der Geruch nach Schwefelwasserstoff verschwunden ist. Das Zinn fällt dabei als Zinnsulfid in der bekannten gelbbraunen Form aus. Der Niederschlag wurde dann abfiltriert und mit Wasser ausgewaschen.

Durch Veraschen wurde das Sulfid in Zinndioxyd überführt und als solches gewogen. Verwandt wurden kleine Porzellantiegel, die in

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einem guten Bunsenbrenner sehr leicht auf Bruchteile eines Zehntel Milligramms konstant erhalten wurden. Die Wägungen erfolgten auf einer Mikrowage von Sartorius. Auf guten Temperaturausgleich wurde großer Wert gelegt. Die Filterasche der verwandten kleinen Filter (rund 3 cm Durchmesser) wog etwa 0,01—0,02 mg. Die Menge war also so gering, daß sie nicht berücksichtigt zu werden brauchte.

Tabelle 2 gibt zunächst einen Aufschluß über die diuretische Wirkung der aromatischen und der aliphatischen Verbindungen des Zinns auf das Kaninchen.

Tabelle 2.

Kaninchen 64, ♀, 1850 g Gewicht: Aromatisches Komplexsalz.

Datum	Gewicht in g	Harnmenge in ccm	Injektion 23 mg Sn/kg am
17. IV.—24. IV.	1850	245	—
24. IV.— 1. V.		280	—
1. V. — 8. V.		340	—
8. V. —15. V.		630	8. V.
			11. V.
			14. V.
15. V. — 22. V.	1945	920	16. V.
			19. V.
			22. V.
22. V. —29. V.		1220	—
29. V. — 5. VI.		1250	—
26. VI.— 3. VII.	2130	960	—
1. IX. — 8. IX.		380	—

Kaninchen 65, ♀, 2250 g Gewicht: Aromatisches Komplexsalz.

Datum	Gewicht in g	Harnmenge in ccm	Injektion 23 mg Sn/kg am
17. IV.—24. V.	2250	280	—
24. IV.— 1. V.		200	—
1. V. — 8. V.		290	—
8. V —15. V.		500	8. V.
			11. V.
			14. V.
15. V. — 22. V.	2225	615	16. V.
			18. V.
			22. V.
22. V. —29. V.		565	—
29. V. — 6. VI.		680	—
26. VI.— 3. VII.	2260	740	—
1. IX.— 8. IX.		300	—

Kaninchen 18, ♀, 2300 g Gewicht: Aliphatisches Komplexsalz.

Datum	Harnmenge in ccm	Injektion 4,5 mg Sn/kg am	Bemerkungen
11. VI. —18. VI.	380	—	—
18. VI. — 25. VI.	400	—	—
25. VI. — 2. VII.	315	25. VI.	29. VI.: Apathisch, frißt kaum.
		27. VI.	—
		29. VI.	—
2. VII. — 9. VII.	220	2. VII.	—
9. VII.—13. VII.	60	—	12. VII.: Tot.

Tabelle 3 enthält einen Auszug aus den Versuchsprotokollen über Aufnahme und Ausscheidung des Zinns (als aromatische Verbindung) bei zwei Kaninchen.

Tabelle 3.

Aromatisches Komplexsalz.

Kaninchen 11, ♀, 1930 g Gewicht					Kaninchen 12, ♂, 2000 g Gewicht			
Harn		Kot		Datum	Harn		Kot	
Menge in ccm	Sn in mg	Menge in g	Sn in mg		Menge in ccm	Sn in mg	Menge in g	Sn in mg
575	5,70	89	10,34	23. II. — 2. III.	600	7,24	62	7,67
1175	6,24	132	18,09	2. III — 9. III.	700	5,18	84	5,92
1210	3,76	51	8,03	9. III.—16. III.	680	2,43	95	5,16 [1]
1210	5,05	100	15,20	16. III —23. III.	900	4,26	116	10,14
900	6,00	—	30,21	23. III —30. III.	850	8,22	—	12,30
450	7,11	170	75,19	30. III.— 6. IV.	980	12,14	105	16,68
580	3,60 [1]	172	23,00	6. IV.—14. IV.	830	16,42	—	20,31
				14. IV.—17. IV.	520	1,41	36	24,07

Kaninchen 11 erhält Injektion: Am 23. II. 34 mg Sn/kg; 26. II., 1. III., 5. III., 8. III., 12. III., 15. III., 17. III., 20. III., 23. III., 25. III., 27. III., 30. III., 1. IV., 3. IV., 5. IV. je 23 mg Sn/kg; 6. IV. 46 mg Sn/kg. † 14. IV.

Sektion: Leber: Fettige Degeneration: in den übrigen Organen, auch in den Nieren makroskopisch und mikroskopisch kein pathologischer Befund. Wochenharnmenge normal: 290 ccm.

Kaninchen 12 erhält Injektion: Am 23. II. 34 mg Sn/kg; 26. II., 1. III., 5. III., 8. III., 12. III je 23 mg Sn/kg. Wochenharnmenge normal: 320 ccm.

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[1] Kleine Verluste.

Kaninchen 11

erhält injiziert . . .	813	mg Sn	23. II.—6. IV.
scheidet aus im Kot.	181,90	» »	
» » » Harn	37,40	» »	
insgesamt	219,30	mg Sn	

Kaninchen 12

erhält injiziert . . .	257	mg Sn	23. II. bis 16. III.	0,00 mg Sn	16. III. bis 13. IV.
scheidet aus im Kot.	16,65	» »		83,43 » »	
» » » Harn	14,80	» »		43,17 » »	
insgesamt	33,45	mg Sn		126,60 mg Sn	

Aus diesen Versuchen geht hervor, daß im Kot wesentlich mehr Zinn ausgeschieden wird als im Harn; wie bei anderen Schwermetallen fällt auch hier die ungewöhnlich langsame Ausscheidung auf; 4 Wochen nach Aufhören der Zinninjektionen werden noch beträchtliche Mengen in Harn und Kot ausgeschieden. Mit dieser langsamen Ausscheidung hängt wahrscheinlich die ungewöhnlich lange Dauer der gesteigerten Diurese zusammen. Die Zeit des Eintrittes der Diurese war verschieden: 8mal nach der zweiten Injektion, 3mal nach der dritten, 3mal nach der vierten Injektion von 23 mg Sn/kg.

Auch am Hund ließ sich mit der aromatischen Zinnverbindung eine diuretische Wirkung erzielen, vgl. Tabelle 4.

Tabelle 4.

Hund, ♂, 6000 g Gewicht.

Datum	Injektion 20 mg Sn/kg am	Harnmenge in ccm	Durchschnittliche tägliche Harnmenge in ccm
7. XI.—16. XI.	—	1445	161
16. XI.—25. XI.	16. XI.	2005	225
	19. XI.	—	—
	23. XI.	—	—
25. XI.—30. XI.	—	1680	336
30. XI.— 9. XII.	—	1395	155

Lokale Reaktionen waren bei den Injektionen des aromatischen Komplexsalzes nicht zu beobachten, die Hunde empfanden die Injektionen schmerzhaft, auch beim Menschen (Selbstversuch) war die Injektion von 1 ccm der 1%igen Sn enthaltenden Lösung nicht sehr, aber immerhin deutlich schmerzhaft.

Selbstverständlich haben wir auch die diuretische Wirkung des Komplexbildners untersucht, konnten aber nie eine Steigerung der

Diurese beobachten. Mit der aliphatischen Komplexverbindung konnten wir (vgl. Tabelle 2) auch keine diuretische Wirkung beobachten, allerdings konnten wir ja nicht annähernd so hohe Konzentrationen verwenden, wie von der aromatischen Verbindung.

Die Nieren der Tiere wurden sowohl auf der Höhe der Diurese (vier Tiere) als auch nach Abklingen derselben (vier Tiere) mikroskopisch untersucht, da der Verdacht einer entzündlichen Reizung nahe lag; die Nieren waren aber stets (acht Tiere wurden untersucht) ohne pathologischen Befund; Eiweiß wurde im Harn nie gefunden.

Die Ursachen der diuretischen Wirkung des Zinns in geeigneter Bindung und Konzentration lassen sich ebensowenig wie die des Quecksilbers näher definieren; im akuten Versuch war, wie vorauszusehen, bei intravenöser Injektion keinerlei diuretische Wirkung zu erzielen. (Uretherenkatheterismus, Einfließen einer 1% Sn enthaltenden Lösung in die Ohrvene: 13,5 ccm in 24 Minuten, 16 ccm in 15 Minuten, 25 ccm in 16 Minuten: ohne diuretischen Erfolg.)

Die eigenartige Verteilung des ausgeschiedenen Zinns auf Harn und Kot veranlaßte uns die Ausscheidungsart anderer Schwermetalle, soweit dies möglich war, aus der Literatur zu studieren. Vom Cu weiß man, daß man eine Ausscheidung im Harn bzw. Kot überwiegen lassen kann, je nach dem Phosphatgehalt und der H-Ionenkonzentration des Blutes. Über die Ausscheidungsweise der Schwermetalle gibt die folgende Tabelle 5 einen Überblick.

Tabelle 5.

Metall	Atomgewicht	Ausscheidung Kot — Harn	Literatur
Al	27	K ≧ H	Kunkel, Toxikologie.
Mn	55	0	Handovsky-Schulz-Staemmler, a. a. O.
Fe	56	K ≧ H	Vgl. Kunkel, a. a. O., S. 176; ferner Kobert, Dorpater Arbeiten VII.
Ni	59	K ≧ H	Kunkel, a. a. O., S. 178; Kobert, Toxikologie, S. 59.
Zn	65	K > H	Kobert, Dorpater Arbeiten IX.
Cu	63	K ≧ H	Kobert, a. a. O.
Ag	108	K > H	Kobert, Dorpater Arbeiten IX.
Sn	119	75 : 25	Handovsky.
Au	197	K = H	Heubner, Dieses Archiv, Bd. 56.
Hg	201	30 : 70	
Pb	207	45 : 55	Lomholt, Biochem. Journ. 1924, Bd. 18, S. 60.
Bi	208	85 : 65	

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Die Angaben aus den Kobertschen und Kunkelschen Toxikologien sind mit denen von Lomholt und uns nicht direkt vergleichbar, da es sich bei ihnen nicht um chronische Vergiftungen an Kaninchen handelt. Darum haben wir nur jene Metalle hier angeführt, bei denen die Autoren ausdrücklich darauf hinweisen, daß bei subkutaner Einverleibung wesentlich mehr in Kot oder Harn ausgeschieden werden.

Jedenfalls glauben wir aus dieser Zusammenstellung schließen zu können, daß die Schwermetalle mit niederem Atomgewicht vorwiegend im Kot, die mit hohem Atomgewicht vorwiegend im Harn ausgeschieden werden. Welche mit der atomistischen Struktur zusammenhängenden Eigenschaften es sind (Oxydbildung, Stellung in der Spannungsreihe), die für dieses eigenartige biologische Verhalten der Schwermetalle verantwortlich zu machen sind, läßt sich nicht sagen. Man könnte daran denken, daß es für eine diuretische Wirkung nötig ist, daß ein wesentlicher Teil des Metalls durch die Nieren ausgeschieden wird; im Zinn haben wir ein Metall vor uns, das in hinreichender Menge durch die Nieren ausgeschieden wird, das aber im Gegensatz zu Hg keine Nephritis macht. Wir haben also ein wirksames und unschädliches metallisches Diuretikum vor uns, allerdings braucht man zur Hervorrufung der Diurese größere Mengen.

Dark Discoloration of Canned All-Green Asparagus

I. Chemistry and Related Factors

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SUMMARY

The classical concept of asparagus discoloration is evaluated. Dark discoloration of all-green asparagus brine was found to be dependent on the type of container corrosion. This is affected by unknown systems in the asparagus itself. Methods of process that modify the corrosion process are discussed. This study led to the conclusion that discoloration does not occur if sufficient stannous tin is in solution. All effects of other factors are related to this one fact.

INTRODUCTION

The development of better containers for food products is increasingly dependent on basic knowledge of the factors underlying any given packaging problem. As food science and technology advance at accelerated rates, sound principles must be used to solve these problems.

Since 1928 this laboratory has been concerned with two major problems of asparagus canning: 1) sporadic brine discoloration, and 2) short service life (American Can Co., 1930). The research reported here was begun in 1951 to expand knowledge gained from earlier work. A coordinated three-phase program was set up:

1) A grant was made by the American Can Company to the National Canners Association for university-level research. This was to determine chemical changes in the raw product under different growing and harvesting conditions. This work has been reported (Dame *et al.*, 1957a,b, 1959a,b).

2) A study was begun, in cooperation with the N.C.A., to determine the effect of processing variables. The N.C.A. has reported on this research (NCA, 1958).

3) An over-all program was initiated to correlate the findings of the first two phases of the program and to apply these directly to work with container variables. Results of this phase are reported here.

When this work was begun, these facts were known:

1) The pH of asparagus brine taken immediately after cutting was usually lower for dark brine than for normal brine (American Can Co., 1930).

2) If 1000 ppm of citric acid was added to the asparagus, the discoloration was prevented (American Can Co., 1930).

3) Rutin had been shown to be present in asparagus (De Eds and Couch, 1948; Stevenson, 1950). Neither of these authors, however, suggested that rutin was involved in asparagus discoloration.

4) Rutin was known to have a dark iron complex, and it was generally believed that the dark discoloration of asparagus was associated with asparagus whose rutin content would be high.

The present study analyzed these previously known facts and extended along five lines of attack:

1) The exact nature of the discolored complex was studied. These studies helped to clarify the role of iron and tin ions.

2) The influence of the initial pH of the asparagus was studied.

3) The role of rutin was analyzed.

4) The effect of different blanching procedures was analyzed. These have been interpreted in terms of the information now available.

5) The mechanism of action of citric acid was investigated.

EXPERIMENTAL

Analysis for rutin and quercetin. The flavone nucleus without a sugar attached is rather insoluble in neutral or acid water, but is soluble in higher alcohols, such as octanol. The glycosides, by virtue of their sugar moiety, are soluble in water. This difference is sufficient that, by carefully controlling the solvent amounts of water-octanol-methanol (3:1:2) the quercetin can be extracted almost totally into the octanol layer while the rutin has a partition coefficient of 93:7 in favor of the water-methanol layer.

Quercetin cyanidin in pure solutions has a broad absorption maximum from 500 to 525 mμ, whereas rutin cyanidin has a broad absorption maximum from 535 to 550 mμ. This makes it possible to analyze for rutin by partition extraction of the compounds after first reducing the sample with HCl and magnesium.

Fifteen ml of clear sample are diluted with 10 ml methanol, and 0.4 ml HCl and sufficient magnesium turnings are added to continue bubbling for at least twice as long as the visual reaction to produce a full red. The water-methanol-HCl-magnesium mixture is extracted twice with 5 ml of octanol. The resulting solution is then analyzed for rutin by determining the transmission at 550 mμ. The octanol phases are then combined, washed twice with 1:1 water-methanol, and analyzed for quercetin by measuring transmission at 500 mμ. By carefully controlling amounts of solvent, standard curves can be easily drawn, and both the amount and type of flavonol determined. As a check on this method, samples were also analyzed for rutin by the $AlCl_3$ shift (Griffith *et al.*, 1955).

Iron analysis was by wet ashing followed by colorimetric determination of the iron with orthophenanthroline.

Tin was determined by the AOAC volumetric method (AOAC, 1940).

In all of the experiments described, except where otherwise noted, the containers used were 211×400 cans with plain common coke tinplate bodies and C-enameled electrolytically coated ends. Whenever possible, all test packs of asparagus were made in commercial canneries. The asparagus was a small-medium blend unless otherwise specified.

In laboratory packs, medium-large blends of fresh asparagus were blanched in stainless-steel colanders by immersing the filled colanders for 3 min in boiling water or for 2 min in an exhaust box. The blanched asparagus was rinsed with cold water and hand packed into cans. The packed cans were brined, exhausted to 190°F can center temperature, closed, and processed for 15 min at 248°F.

RESULTS AND DISCUSSION

Basic chemistry of rutin. To interpret the other results, tests were made to study the general reactions that rutin might be expected to undergo when subjected to the pH, tempera-

Table 1. Dissolved metal and discoloration.

Cannery	Initial pH[a]		Av. pH	Five months' cutting		
	Range	Av.		Samples discolored (%)	Av. tin/can	Av. total tin (ppm)
A	5.50–5.63	5.53	5.41	44.7	.17	60
B	5.43–5.64	5.53	5.41	18.7	.63	73
C	5.15–5.85	5.65	5.45	7.5	2.65	64
D	5.35–5.95	5.59	5.34	3.1	5.5	116
E	5.35–5.75	5.67	5.37	1.8	8.3	147

[a] Initial readings were of 10 or more containers for each lot. The readings were made on the 10th day after packing.

tures, and ionic environment it might encounter during the standard asparagus sterilization process.

Influence of pH and temperature on solubility of rutin. The solubility of rutin in neutral solution has been reported (Krewson and Naghski, 1952). The solubility of pure rutin in 70°F water at neutrality is less than 20 mg/100 ml. To determine the effect of pH, a solubility curve was run for pH 3 to pH 9 (Fig. 1). This showed that the influence of pH on the solubility of rutin is negligible in the range normally encountered in canned asparagus.

We run the temperature-solubility curve of rutin in water at pH 6.0, the approximate pH of freshly canned asparagus brine. We confirmed the work of Krewson and Naghski (1952). The curve showed that it is easy to dissolve high concentrations of rutin, but within 24 hr the excess of dissolved rutin has precipitated. This is the reason for the appearance of a rutin precipitate in glass-packed asparagus.

The amount of rutin in the brine of canned asparagus often runs above 150 mg/100 ml. It is clear that neither of the above factors can account for this amount; therefore it is necessary to look for other factors.

Influence of tin and iron on solubility. The solubilizing effect of iron salts on rutin was investigated by Krewson and Couch (1952). To evaluate this and the effect of dissolved tin, rutin was processed in glass tubes that contained either tin or iron strips. Ten ml of H_2O and 200 mg rutin were placed in 10×300-mm pyrex tubes. The metal strips of tin or iron added were $\frac{3}{8}$×2×0.012 in.

The solubility of rutin in distilled H_2O, without metal addition, is 15 mg/100 ml after 15 min at 218°F and 24 hr at 70°F. The solubility with added tin strips was 38 mg/100 ml under the same conditions, and the solubility with added black iron strips was 158 mg/100 ml.

These data explain why it is possible to obtain more rutin in solution in canned asparagus than in simple buffered solutions.

Titration curve for rutin. The sharp solubility change of rutin between pH 7 and pH 8 suggests a titratable acid group. It was felt that it might be of interest to know the status of the titratable acidic groups of rutin. Fig. 2 shows the titration curve of pure rutin. There are three titratable acid groups with pH values of 1.0, 2.5, and 7.5. During the titration, rutin was found to be an acid-base indicator, as can be seen from the color changes indicated in Fig. 2.

Hydrolysis of rutin. One of the questions that arises is whether rutin is hydrolyzed into quercetin during the process. Solutions of 40 mg/100 ml stannous rutinate adjusted in pH from 3 to 7 in citrate or phosphate buffer were autoclaved in glass tubes for 15 min at 248°F (standard process for asparagus in No. 2½ and smaller cans). The pH was checked before and after processing. Fig. 3 shows the analysis for rutin and quercetin. Hydrolysis of rutin to quercetin is apparently maximum at about pH 6.0. The changes described here probably represent straight hydrolysis, because spectral analyses of the mixtures with a Beckman DU spectrophotometer, before and after, show only the peaks typical of rutin and quercetin.

Color of discolored asparagus brine. When a can of asparagus that is going to show severe "tannate" discoloration is opened and the brine immediately poured to a depth of ¼ inch in a white pan, streaking appears in about a minute and increases to a maximum severity at about 15 minutes. The color of the brine is often described as black, but closer examination reveals a green tinge to the "black." After standing 3–4 hr, the color changes to a black with little or no greenish tinge.

A sample of asparagus that is to show less severe discoloration will have streaking after 3–5 min. with maximum darkening after 15–20 min. The color at this time is green-black. After 3–4 hr, however, this color fades and is replaced by brown.

It has been postulated that discoloration is initiated by oxidation of the ferrous ion. The ferric ion then combines with rutin or a rutin complex, causing discoloration (Davis *et al.*, 1961).

If one tries to duplicate these reactions in buffered systems, severe problems are encountered. An unbuffered solution of rutin and ferric chloride has a greenish-brown color that is primarily green. The pH of a 40 mg/100 ml solution of rutin-$FeCl_3$ (1:1 molar) is 2.8. When 0.1*M* acetate or 0.1*M* phosphate buffer at pH 5.5 is used to dissolve the 40 mg/100 ml rutin-$FeCl_3$, the color is a clear brown. If, however, dilute $FeCl_3$ is added dropwise to a 10 mg/100 ml solution of rutin at this pH, the color first has a greenish cast and then, as the concentration of iron increases, becomes brown. If the brown solution is heated, the color again becomes the greenish black of a typical "tannate" discoloration.

Dropwise addition of 1% H_2O_2 to the unbuffered greenish solution produced by heating $FeCl_3$ and rutin at pH 2.8 returns the solution to a clear brown, and further addition bleaches out the color.

If one produces a pure ferrous ion by boiling iron wire in 0.1*M* acetate buffer at pH 5.5 under a nitrogen atmosphere and then adds a few mg of rutin, an intense green is produced. This color is so intense (almost black) that as little as 2 mg/100 ml of rutin will have no transmission in a 1-cm spectrophotometer tube. The greenish black has somewhat the appearance of "tannate" discoloration.

When ferric chloride is added to quercetin at pH 5.5 in 0.1*M* acetate

Table 2. Container variables and cutting data for asparagus packed on fourteen different days at the same cannery.

Lot	Description	Average pH: Initial	3 mo.	7 mo.	10 mo.
1	Type BR	5.76	5.71	5.47	5.30
2	Type L	5.77	5.60	5.45	5.23
3	Type L	5.76	5.68	5.46	5.35
4	Lot 2 cathodically cleaned	5.76	5.62	5.45	5.23
5	Lot 3 cathodically cleaned	5.76	5.62	5.44	5.24
6	Lot 5 anodized	5.77	5.66	5.48	5.34
7	Scratched C-enameled bodies	5.79	5.74	5.51	5.43

Tops: C-enamel.
Bottoms: C-enamel.
Bodies: All are plain common coke except Lot 7 which is C-enamel.
Can size: 211 x 400.

Table 3. pH and discoloration grades.

Lot	Av. initial pH	Av. pH after 10 mo.	Discoloration grade* after 10 mo.: Range	Av.
2	5.77	5.23	0–1	0.3
4	5.76	5.23	0–1	0.4
5	5.76	5.24	0–1	0.2
1	5.76	5.30	0–0.5	2.7
4	5.77	5.34	1.5–8	3.8
3	5.76	5.35	0–5.5	3.2
7	5.79	5.43	9–10	9.7

* The following grading system was used:
0 = No discoloration, clear yellow.
1–3 = Yellow with increasing brownish tinge.
4 = First trace of transient dark streaks on surface of brine.
5–6 = Definite streaking, increasing dark tinge.
7–9 = Noticeable dark discoloration of increasing intensity.
10 = Extreme discoloration; brine is black with green tinge; product is slate green.

rosion pattern for the various lots led to different amounts of dark discoloration. A further substantiation of the premise that the predominance of iron corrosion over tin corrosion is the cause of discoloration is seen in lot 7 (the scratched fully enameled container). With this type of container the corrosion of iron predominates. Note that all lots discolored, and the pH decrease was the least in lot 7.

From these data one might well ask if the containers were not the only factor concerned in the problem of dark discoloration. Table 1 gives the results of packing one lot of plate at five different canneries. Note that different lots of asparagus tend to change the corrosion pattern so that the discoloration varies substantially although the initial pH values are essentially the same. The pH values after storage do not align themselves according to the more classical concept of discolored asparagus brine falling above pH 5.6 and normal falling below 5.6. They do show that the amount of pH change is directly related to the degree of discoloration. This correlation is also shown in Fig. 4, where the pH decrease is plotted against the average discoloration grade. Lot 1, which does not appear to fall on the curve, is out of location because of erratic performance in which one or two cans showed intense discoloration. The result is that although the pH of these samples themselves showed a little change, they are not sufficient to significantly change the average pH reduction, but are sufficient to change the average discoloration grade.

The role of rutin. Since the rutin complexes of iron are dark in color, it is an attractive postulate that the dark discoloration of canned asparagus consists at least partially of these iron-rutin complexes. However, even though the dark material is probably an iron-tannin complex, addition of rutin itself does not increase the discoloration. Rutin is therefore not a limiting factor. Components other than rutin seem to be more involved in the causative mechanisms of discoloration than is rutin itself. These components affect the corrosion mechanism and thus the ultimate discoloration.

Table 4 shows the effect of rutin addition on the discoloration obtained in two test packs.

Table 4. Effect of rutin on discoloration grade in 211 x 400 containers.

Pack	Rutin added (mg/100g)	No. cans	Av. pH	Av. dis. grade
D	0	4	5.25	0
D	33	4	5.20	0
D	85	4	5.20	0
S	0	15	5.46	0.6
S	60	15	5.43	0.1

It is more difficult to substantiate the conclusion that the discoloration system is concentrated in the tips. However, test packs of asparagus made over the years have shown that asparagus tips have a far greater tendency to discolor than do center cuts.

Effect of water blanch and steam blanch. Water blanching makes asparagus more corrosive toward tin than does steam blanching. This type of corrosion does not result in discolored asparagus. Table 5 shows the results of a test pack to determine the effect of water blanching and steam blanching. Since it was previously determined that rutin is not the limiting factor in discoloration, it is obvious that the changes are in the extraction or modification of components whose identities are not presently known.

Table 5. Effect of blanch on discoloration grades.

Type blanch	No. of cans	Av. discoloration grade	Tin-coating attack
Steam	40	6	No etch on container
Water	40	1	Containers heavily etched

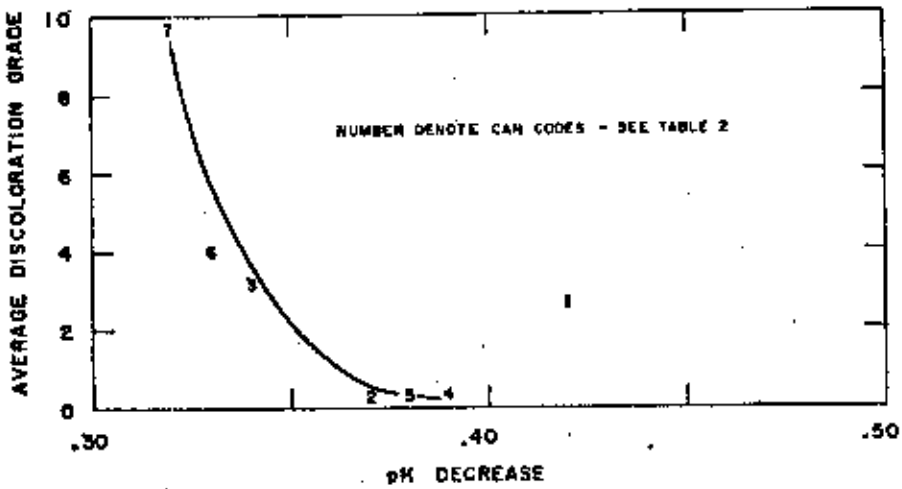

Fig. 4. Decrease of pH of all-green asparagus brine during storage as related to discoloration grade.

Table 6. Effect of added citric acid on container corrosion and product texture.

Pack	Citric acid added (ppm)	Texture	Container corrosion
177-2A control	0	Normal	Light etch
177-2A citric	250	Softer with more sloughing	Moderate etch
177-15A control	0	Normal	Light etch
177-15A citric	250	Softer with more sloughing	Moderate etch

The mechanism of action of citric acid. Citric acid can be used to reduce the severity of brine discoloration (American Can Co., 1930; NCA, 1958; Davis *et al.*, 1961). The most likely mechanism for this would be its effect of chelating tin, thus increasing the electrochemical protection of the base steel. A second possibility might be the chelation of iron, thus augmenting the mass-action elimination by tin of iron from the colored complexes (Davis *et al.*, 1961).

Experiments since 1955 with the addition of citric acid have been plagued with a problem known by all workers in the field. It is necessary to pack an asparagus that discolors before an additive can be shown to be beneficial. None of the control lots in this test discolored. However, other workers have established this point beyond reasonable doubt, so primary interest was in the mechanism of action as well as an analysis of its relative advantages or disadvantages.

Table 6 demonstrates the effect of 250 ppm of added citric acid on container corrosion. The assumption that the primary effect of citric acid would be on its ability to increase the anodic protection of iron by tin is borne out by the increased etch where citric is added. Table 7 also bears out this

Table 7. Effect of added citric acid on vacuum (10-oz. cutting).

Citric acid added (ppm)	Av. vacuum (in.)	Av. head-space (32nd in.)	Av. pH	Av. discoloration grade
None	8.1	10	5.21	0
50	9.7	11	5.19	0.1
50	11.2	11	5.18	0
100	11.5	11	5.16	0

finding, since the loss of vacuum in the second pack is shown to be lowered by citric addition. This loss of vacuum is most probably associated with the corrosion of the base steel. Unfortunately, the brine from these containers was not analyzed for tin or iron. However, all three factors that accompany citric addition (higher vacuum, lower pH, and greater etch) are usually associated with a greater anodic protection of iron by tin, and thus a more favorable tin-iron ratio.

The use of citric might be more encouraging if it were not for the fact that some softening of the asparagus occurs (Table 6). The asparagus also shows some change in flavor (Table 8).

Table 8. Triangular taste tests to ascertain effect of added citric acid.

Pack No.	Citric acid added (ppm)	No. of tasters	No. of tasters detecting no difference
177-2	0 and 83	7	5
177-2A	0 and 250	7	3
177-15	0 and 83	7	6
177-15A	0 and 250	7	3
303-12	0 and 50	8	8
303-12A	0 and 50	8	3

At the five months' examination, asparagus from cans to which 200 ppm (of total can contents) of citric had been added could be separated by flavor from cans to which it had not been added. This flavor difference was not necessarily unacceptable, however. Several of the tasters stated a preference for the cans with added citric acid.

The data presented have led to a much different concept of the problem of dark discoloration in asparagus. It is now possible to shift attention from the symptoms of the problem to the causes of the problem. Control of the pattern of corrosion would seem to be the key to the prevention of dark discoloration in asparagus.

Citric addition does not appear to be an acceptable solution. The sloughing and fragility of asparagus is enough of a problem without compounding it by an additive that increases the sloughing tendency.

In these tests it was not possible to determine the exact nature of the discoloration pigment, nor could the fact be explained that air is required before the black streaking occurs. Similarly, no attempt was made at this time to prove the mechanism of the effect of citric acid on container corrosion. It is not yet known what organic systems are responsible for changing the corrosion pattern of canned asparagus. Further study of corrosion mechanism is planned. It is in this field that the least usable information is available at the present time.

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THE EFFECTS OF METALLIC SALTS ON THE HISTOLOGY AND FUNCTIONING OF THE RAT TESTIS

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Summary. A series of experiments was carried out to determine the effect of five metals on the histology and functioning of the testis and epididymis in rats. The metals (silver, copper, tin, nickel and cobalt) were administered subcutaneously as aqueous salts in a single dose or as daily injections from 1 to 30 days; the testes were then subjected to normal histological examination and dark ground microscopy, and to radiography following intra-arterial injection of Micropaque in one experiment.

All five metals produced acute and chronic changes in the histology of the testis and interfered, to some degree, with spermatogenesis. All tissues showed improvement following the initial injection, even in spite of continued daily injections. The individual results are described in detail.

The effects and possible modes of action of these metals are discussed and contrasted with cadmium. It would appear that all the metals investigated act in a different way from cadmium, and may in fact limit their own action by precipitating proteins in the membrana propria, thus making it impermeable to further metallic ions. It is possible that the action of the metals on the epididymis varies from that on the testis, and the damage produced in the epididymis and ductuli efferentes shows less tendency to recover.

INTRODUCTION

Since Pařízek first investigated the effects of cadmium on the histology and functioning of the testis in 1956, much work has been done on the various aspects of its action by Pařízek and others.

Relatively little work has been published on metals with a similar chemical activity to cadmium in their action on the testis. Kamboj & Kar (1961) investigated a large number of metallic and rare earth salts, and some aspects have been recorded of the effects of lithium (Mackeod, Swan & Aitken, 1949) and selenium (Maekawa, Tsunenari & Kurematsu, 1965).

It would appear necessary, therefore, to make a thorough investigation of the action of metallic salts, closely related to cadmium in the Periodic Table, on testicular and associated tissues, and spermatogenesis. In the present work silver, copper, tin, nickel and cobalt salts are investigated.

MATERIALS AND METHODS

Male albino rats, selected at random, were used. They were housed under identical conditions for the duration of the experiments, and killed by means of an overdose of chloroform.

The testes were removed at death and fixed in Bouin's solution (unless otherwise indicated in the description of the experiments). Serial sections of the upper half of the testis, the rete testis, ductuli efferentes, ductus deferens and the head and body of the epididymis were prepared and stained by means of iron haematoxylin and eosin. Unstained sections were also prepared to permit of dark ground microscopy.

Aqueous solutions of the salts of five metals, namely silver nitrate, copper sulphate, nickel sulphate, cobalt chloride and stannous chloride were administered by subcutaneous injection, the dose being 0·01 m-mole/kg body weight. Any variation or addition to this procedure will be described with the relevant experiment.

The following experiments were performed:

(1) Five rats weighing from 160 to 170 g were used. Each rat was injected, according to weight, with a different metal. The rats were then killed 18 hr after injection and the testes removed and treated as above.

(2) Five groups of rats, with four rats in each group, weighing from 120 to 200 g, were given daily injections of one metal. A rat from each group was killed at 2, 10, 21 and 30 days, respectively, after the first injection.

(3) Five groups of rats, three rats in each group, weighing from 206 to 270 g, were given a single injection of one metal. A rat from each group was killed 4, 8 and 12 days after injection respectively.

(4) Five groups of three rats, weighing from 140 to 315 g, were given ten daily injections of one metal. A rat from each group was killed the day after injections ceased, then one on the 4th and one on the 8th day.

(5) Five rats, weighing from 120 to 156 g, were injected with one of the metals. Twenty-four hours later each rat was anaesthetized, the thoracic cage was opened to expose the thoracic aorta, and 25 ml of Micropaque (Damancy & Co.) were injected into the aorta, which was then ligated. The testes were removed and X-rayed, using Kodak RS 5 plates.

Tubular diameters were calculated as the mean of the shorter diameter of a random sample of not less than fifty tubules.

RESULTS

At the outset of the experiments it was found that there was uniformity of results and the sequential changes observed demonstrated a progressive series. It was, therefore, considered unnecessary to repeat the experiments using more animals.

Every set of experiments produced changes in the testis and adnexa. To the naked eye the testes appeared normal in size and colour as regards the tissue itself, but hyperaemia was more or less marked in all cases, and those specimens

exhibiting pronounced hyperaemia frequently had small punctate haemorrhages in the fatty body.

The various histological changes will be described in detail and the individual results will then be related to these changes.

ACUTE CHANGES

These were manifest in Experiment 1.

The most marked change is shrinkage of the tubules, particularly the more centrally placed ones (mean diameter = 16·9 μ), to give wide spaces between the tubules in which the isolated interstitial tissue is found. The peripheral tubules retain their normal diameter (mean diameter = 19·3 μ), and though the depth of these normal tubules varies from one to four, the area is generally uniform around the testis (Pl. 1, Fig. 1).

Eosinophilic oedema fluid is always present in the spaces and is most marked in the peripheral area. There is a great increase in the lymphatic drainage, all the vessels being grossly enlarged, and in some cases they contain the breakdown products of haemoglobin. Similarly there is hyperaemia with marked engorgement of the intertubular capillaries, and of the testicular veins particularly.

Within the tubules the damage varies in extent, being most marked in the central tubules. Many of these are grossly shrunken (to as little as 14 μ in diameter) and have their central lumen entirely obliterated, whilst the tubular tissue is darkly stained and the cells no longer well differentiated. Gradation of damage from this to normal occurs and in some tubules, commonly the central ones, groups of cells undergoing mitosis are visible (Pl. 1, Fig. 2).

The interstitial tissue shows considerably less damage than the tubules at this stage. It is normal in amount and may be quite normal structurally. Where damage does occur the nuclei of the Leydig cells show thickening of the nuclear membrane and clumping of the chromatin and, when present, the damage occurs throughout the whole testis (Pl. 1, Fig. 3).

Spermatogenesis does not appear to be affected, apart from increased nuclear basophilia of spermatogonia and Sertoli cells. The formed spermatozoa, however, frequently show separation of the head and some tubules have a ring of tailless, pyknotic sperm heads around the lumen. The condition of the spermatozoa in the epididymis varies from normal to quite degenerated.

The ductuli efferentes show marked damage, with considerable epithelial proliferation, and areas of complete blockage. This can be seen to produce some enlargement of the proximal ductuli and of the rete testis (Pl. 1, Fig. 4).

Changes in the epididymis vary from negligible to marked, particularly in the head of the epididymis which may contain shrunken and distorted tubules showing patchy secretory activity (Pl. 1, Fig. 5).

Experiment 1

This showed the acute changes occurring 18 hr after a single injection.

Silver. This will be taken as the standard metal for comparison with other metals. Silver, in fact, produced marked damage to all the tissues; even the

peripheral tubules were affected, and some central tubules had completely disintegrated, leaving a space ringed by interstitial tissue, which also showed damage. There was very marked deformation of the tubules in the head of the epididymis, whose epithelial cells appear swollen because of the tubular shrinkage.

Copper. Copper shows the same range of damage but to a lesser degree, and a normal peripheral band, four tubules deep, remains. The head of the epididymis is relatively normal, apart from the secretory activity. This metal causes spermatogenic damage of the nature described above in the central third of the testis.

Tin. Tin resembles silver closely, but cellular differentiation is retained. Interstitial tissue damage is greater than with copper. Epididymal damage resembles copper, but the spermatozoa here are markedly degenerate.

Nickel. This resembles silver without the interstitial damage. There is some shrinkage of the epididymal tubules but their regular shape is retained. The spermatozoa in the epididymis are completely degenerated.

Cobalt. Cobalt has no effect on the interstitial tissue but the deformation of the epididymal tubules resembles silver. Spermatogenesis is as active as with silver, but abnormal heads are more predominant.

Dark ground microscopy in all cases shows that the metal is present in the interstitial tissue, the lumina of all the tubules (in the spermatozoa), sometimes in the tissue of the peripheral tubules, and in the spermatozoal contents of the epididymis. Occasionally it occurs in the tunica albuginea. Copper occurs in smaller quantities than silver, as does nickel. Tin is present in similar amounts to silver, and cobalt is intermediate between the two groups.

CHRONIC CHANGES

These were seen in Experiments 2, 3 and 4. In most cases the predominant tendency is for the tissues either to recover or to deteriorate, but both processes may occur in the same testis: some tubules regaining a normal appearance whilst neighbouring tubules degenerate completely.

In the process of recovery the normal tubular size is regained and cell differentiation becomes complete. The lymphatic drainage resumes normal proportions, but it is of interest that, even when the cellular appearance is normal, oedema fluid is still to be found, particularly between the peripheral tubules. Also, the recovery of testicular structure does not necessarily denote a return to normal spermatogenesis and vice versa (see below).

Two distinct types of degeneration occur. The more severe type is an extension of the acute changes. The tubules become more shrunken (to as little as 9·6 μ in diameter) and darkly staining, individual spermatogenic cell types becoming indistinguishable, the nuclei of primary spermatocytes undergoing karyorrhexis and the nuclei of other cell types, apart from Sertoli cells, showing karyolysis. Finally, a more or less homogenous mass results, which is ultimately absorbed or disappears, leaving a space ringed by interstitial tissue. This would appear to be a slower version of the tubular disappearance seen in the acute changes (Pl. 1, Fig. 6).

The less severe degeneration appears to start with single or multiple vacuolation in the tubular tissues, due to isolated death of primary spermatocytes or of Sertoli cell nuclei (Pl. 1, Fig. 7). The damage progresses to a stage at which there is fragmentation of tubular contents (Pl. 2, Fig. 8). This effect appears to be produced by the disintegration of the Sertoli cell cytoplasm. The Sertoli cell nuclei appear swollen and granular and, in places, can be seen being extruded through the membrana propria, often in large numbers (Pl. 2, Fig. 9). The remaining cells in the seminiferous tubules are normal, and groups of them can be seen to undergo mitosis.

The spermatozoa, lying in the tubular lumina in this last group are usually so markedly damaged as to be a homogenous mass. Pyknotic sperm heads are seen in some tubules, commonly ringing the lumen, and devoid of tails. Normal sperm heads, again tailless, occur, lying radially in the tubular tissue.

Full recovery of the duct systems seldom occurs. In almost all cases the ductuli efferentes show marked damage, amounting to blockage of the duct lumen. This can lead to very marked dilatation of the rete testis and proximal ductuli, and collapse of the distal ductuli. The head and body of the epididymis show a more marked ability to recover, necrosis here being rare.

As with Experiment 1, the results of the following experiments will be described with silver as the standard metal. The results of dark ground microscopy will be noted after each experiment.

Experiment 2

This experiment is an attempt to determine the sequence of events occurring when the testis is exposed to metal over a prolonged period, in this case 30 days.

Silver. After 2 days the changes are similar to those in the acute experiment. A band of peripheral tubules (some four to five tubules deep) appears normal but the central tubules are shrunken (mean diameter 16·12 μ) and some show a slight degree of fragmentation of tissue, between well differentiated cells. Mitotic figures occur throughout the testis. There is some folding of the membrana propria of tubules with both apparent and real extrusion of tubular contents (Pl. 2, Fig. 10). Normal and pyknotic sperm heads are present and the interstitial tissue shows some damage, most marked in central areas. The rete testis shows marked dilation resulting from necrosis and blockage of the ductuli efferentes. One tubule in the head of the epididymis is blocked by necrosis.

At 10 days, improvement has occurred and the tubular diameter is increased (to a mean diameter of 19·11 μ), whilst the damage to the interstitial tissue is less marked. Little change has occurred in the duct system. Spermatozoal heads are less numerous in the seminiferous tubules and the majority are pyknotic.

At 21 days, the tubular diameter is normal but many tubules show fragmentation and most of these contain no spermatozoa. Spaces indicating tubular disintegration are present. The few tubules which appear normal contain pyknotic heads around their lumina. The interstitial tissue is normal, but oedema fluid is present in large amounts. The epididymal epithelium has breaks in its continuity, but otherwise the duct system shows little change.

By 30 days normal spermatogenesis has been resumed by many tubules. The cellular structure of the tubules is almost normal. The ductuli efferentes

have recovered to a normal appearance except for a small area very close to the testis. Lymphatic dilatation is diminished although not yet of normal dimensions.

Copper. In the early stages, copper is more destructive than silver. There is no evidence of spermiogenesis, and improvement is less rapid and extensive than with silver. Spermiogenesis improves although it has not fully recovered in 70% of tubules by 30 days. Necrosis of the ductuli efferentes persists, causing gross dilation of the rete testis.

Tin. This produces a more severe chronic damage very rapidly, and spermiogenesis is almost suppressed in the early stages. Necrosis occurs in the head of the epididymis and the ductuli, and leucocytic infiltration occurs between the tubules. The ductuli efferentes contain oedema fluid (Pl. 2, Fig. 11). By 30 days all tubules display normal spermatogenesis up to and including normal spermatozoa.

Nickel. Nickel produces much less testicular damage in the early stages than silver, but the effect on spermatogenesis is similar. The effect on the duct systems resembles tin. Damage to interstitial tissue and the body of the epididymis increases whilst testicular recovery is occurring, and finally full spermatogenesis is resumed while the ductuli remain necrotic.

Cobalt. Cobalt produces a complete range of chronic damage early, and spermiogenesis is almost completely suppressed. The interstitial tissue is damaged and fragmented in places. Necrosis is present only in the ductuli. Some improvement occurs but the necrosis in the duct system increases, and at 21 days there is still shrinkage of tubules in the central third of the testis and spermiogenesis is only present in 48% of tubules.

Dark ground microscopy shows a similar appearance with all metals. The metal occurs in the interstitial tissue, and in the spermatozoa in the tubular lumina and epididymis, in almost all cases. It is also marked in the blood and lymphatic vessels. It spreads into the membrana propria, the tunica albuginea and the epididymal epithelium and may be cleared out of these latter tissues. Where fragmentation is occurring, in the seminiferous tubules, the metal lines the tissue spaces, so appearing to be present in the tubular tissues. A constant feature at 21 days is the apparent swelling of the membrana propria which although showing little metal appears as a wide shadow (Pl. 2, Figs. 12 and 13).

Experiment 3

This was designed to determine the extent of recovery over a period following a single injection.

Silver. At 4 days, the damage present is much less than at any stage in Experiment 2. Small areas of fragmentation occur in a few central tubules, and normal spermiogenesis is occurring in 60% of tubules, although some pyknotic heads are being produced. There is very marked dilation of the lymphatics, and oedema fluid is present in large quantities. Proliferation of the ductuli efferentes has occurred and the epididymal spermatozoa show degeneration.

At 8 days, some of the testicular tubules show severe chronic damage and others have patches of damaged tissue. The oedema fluid is diminished and the lymphatics are therefore not as dilated. Spermiogenesis has decreased.

being present in only 24% of tubules, and a greater proportion of pyknotic heads is present. The ductuli efferentes show necrosis.

At 12 days, mild chronic damage persists and oedema fluid is still quite prominent. Spermiogenesis is still suppressed, occurring in 62% of tubules and only the peripheral tubules show normal activity. Pyknotic sperm heads are in the majority. The interstitial tissue is normal. Necrosis persists in the ductuli with resultant proximal swelling. The epididymis is normal.

Copper. This produces much more severe chronic damage than silver, and normal spermiogenesis occurs in only 30% of tubules. Necrosis extends into the head of the epididymis as well as the ductuli. Improvement in testicular tissue occurs, but there is only a 10% improvement in spermiogenesis, and necrosis persists in the duct system.

Tin. Initially this resembles copper but spermiogenesis recovers to a greater extent. Nerrosis persists in the ductuli and the epithelial cells of the epididymis are interrupted. Finally, however, the central tubules do not recover full spermiogenesis.

Nickel. Nickel closely resembles silver but spermiogenesis is more severely affected in the early stages, being present in only 16% of tubules, and the condition of the tissues regresses until spermiogenesis has virtually ceased (4%). Some recovery occurs finally, but spermiogenesis remains very limited.

Cobalt. Initially cobalt resembles silver, but necrosis in the ductuli efferentes becomes marked. Spermiogenic activity remains normal throughout.

Dark ground microscopy shows that the metals are present in the same situations as in Experiment 1. Copper is present in smaller quantities than the other metals. At 8 and 10 days the amount of metal present appears to increase.

Experiment 4

This experiment shows the extent of damage and recovery of tissue after a short course of injections.

Silver. At 4 days the histology is nearly normal. Spermiogenesis is occurring in all tubules but many pyknotic heads are present. There is slight interstitial damage and necrosis occurs in the ductuli efferentes.

At 8 days, the overall picture has regressed, with marked degeneration of the central tubules and tissue breakdown in the others. The amount of oedema fluid has increased. Spermiogenesis only occurs in the outermost tubules. Interstitial damage has increased, and in the duct system necrosis is more pronounced and the epididymal epithelium is broken down.

By 12 days recovery has taken place and the picture resembles that at 4 days. Spermiogenesis is more active centrally, and is normal at the periphery. Necrosis persists in the ductuli, and damage to the epididymis is unchanged.

Copper. Copper produces, at 4 days, some mild chronic damage to tubular tissues and spermiogenesis is much reduced. Necrosis occurs in the ductuli efferentes. The epididymal spermatozoa are almost completely degenerated. Again regression occurs, bringing spermiogenesis to a standstill at 8 days, and the final picture, although showing some improvement, is mainly one of mild chronic damage.

Tin. Initially this resembles copper but, although some tubules regress, many

show a rapid improvement; spermiogenesis is quite active by 8 days, and finally is almost normal (90% of tubules) although some pyknotic heads are present in a few tubules. The final picture shows very marked hyperaemia and gross dilation of the lymphatics, although the tissues show only mild damage.

Nickel. Nickel has a similar effect to silver, but the suppression of spermiogenesis is far less than with other metals. Necrosis of ductuli is progressive as with other metals, but the testicular tissue and spermatogenesis are virtually normal by 12 days.

Cobalt. This produces only mild testicular damage initially, and spermiogenic activity is good, about half the formed spermatozoa being normal. Hyperaemia is marked, as with tin. Some interstitial damage occurs and the ductuli show necrosis. Regression occurs which is not much improved by 12 days, although spermiogenesis returns almost to normal.

Dark ground microscopy shows the usual variation in distribution within the tissues. Once again copper is present in less quantity, and at the intermediate stage in the results there is an increase in the amount of metal present in the tissues.

Experiment 5

The results of this experiment are shown in Plate 3.

Silver. This shows clearly the gross enlargement of the lymphatic channels, and the hyperaemia leading to haemorrhages into the fatty body. The vessels displayed in the testis itself are mainly venous.

Copper. Arterial enlargement in the testis is more pronounced and the lymphatic drainage is much less marked than with silver. Haemorrhages are small and scanty.

Tin. Tin combines the properties of both previous metals in its effect. Arteries, veins and lymphatics are all prominent, but haemorrhages are not as marked as with silver.

Nickel. Nickel has a similar but less pronounced effect than tin.

Cobalt. Cobalt is notable for the numerous small haemorrhages throughout the fatty body, although the testicular vessels are not unduly prominent. Lymphatic channels are again much enlarged.

Silver, tin and cobalt show the greatest enlargement of lymphatic channels draining the testis.

DISCUSSION

Although the histological changes produced by the metals under investigation are basically similar to those produced by cadmium (Pařízek, 1957), they occur to a much lesser degree, and none of these metals produced ultimate necrosis and death of the testicular tissue.

The histological changes in the seminiferous tubules differ from those produced by cadmium, in the mature rat, in the degree of shrinkage which occurs. All five metals produce marked shrinkage of some tubules. The fact that neighbouring tubules remain normal excludes the possibility of an artefact. The

concentration of all salts used was similar to that of cadmium as used by Parizek; therefore it would appear that a straightforward osmotic phenomenon is unlikely, but the mode of action could possibly be a manifestation of a Donnan equilibrium across the membrana propria, the membrana being more selective to certain ions. This implies that cadmium may pass across the membrane more readily than the other metals.

Another possibility is that the metals used precipitate proteins, in the membrana propria, more easily than does cadmium, so causing a change in its permeability and achieving their effect by virtue of dehydrating contents of the seminiferous tubules, whilst cadmium may exert a selective toxicity on spermatogenic cells after passing readily through the membrana propria. The concentration of metal in the membrana propria evident in dark ground illumination, together with the swelling apparent in the later stages of Experiment 2, would imply a particular involvement of the membrana.

Both cadmium and other metals probably damage vascular endothelium within the testis, so explaining the increase in interstitial fluid, some of which may be contributed from fluid within the seminiferous tubules in the case of the metals other than cadmium: the changes in vascularity and lymphatic drainage are a logical consequence of this, to remove the excess interstitial fluid. The acute changes in these experiments would seem to be largely due to this mechanism, producing little change in the cellular appearance of the testicular tissue apart from the oedema.

The more prolonged effect of metal administration is interesting in that it produces unmistakable damage to the Sertoli cells. Since these cells are probably responsible for passing nutrition from the membrana propria to the maturing spermatozoa, it is logical that they should be the first cells to be exposed to the metal, and would explain why the spermatozoa, but not the spermatogenic cells, take up the metal so readily. This fact, together with the breakdown and disappearance of the Sertoli cell cytoplasm, is particularly noticeable under dark ground illumination, their position in the tubules being outlined by the metal (Pl. 2, Fig. 12). It is at this stage of Sertoli cell destruction that the membrana propria appears to swell, so it is feasible to hypothesize that the histological improvement commonly seen in the last stage of Experiment 2 may be brought about by the resulting impermeability of the membrana to the metal, so allowing for recovery and regeneration of the Sertoli cells. Since the recovery occurs in spite of continued injections it is obvious that some resistance occurs.

A notable feature of all the metals used is their inability to produce irreversible damage to the testicular tissue. Whereas with cadmium the seminiferous tubules are completely destroyed by a single injection, and the interstitial tissue only shows signs of recovery after 20 days, recovery, to a greater or lesser extent, occurred with all the metals used. However, the degree of damage and recovery with the individual metals varies considerably. Without doubt the metal producing the most lasting damage to both tissues and spermatogenesis, in all the experiments performed, is copper. Silver produces relatively mild damage, and although recovery is not complete in any experiment, it occurs to a considerable degree. Tin and nickel, in general, allow of a good degree of recovery in all cases, despite profound initial damage resulting from tin, and cobalt

produces long-term damage to both tissues and spermatogenesis, whilst recovery after it is generally good in short-term experiments. It is difficult to postulate any reason for the variety of effects found with these five metals; their pH, molecular size and valency are similar and therefore do not appear immediately to have any particular significance, so their ultimate action in the cells must presumably be a purely chemical one.

Whilst the testicular damage is somewhat variable, the damage to the ductuli efferentes and epididymis seems to be more consistent. It may be noted that the epididymis appears fairly resistant to damage, even when dark ground illumination shows the presence of metal at the base of the epithelial cells. Copper is the only metal consistently causing some necrosis of the head of the epididymis—the others produce proliferation and blockage of the ductuli only.

One significant fact is that in Experiment 4, after a short course of injections, the subsequent damage to the whole of the duct system appears to be progressive to a greater or lesser degree.

The question now arises as to the mode of action of the metals on the duct system. Gunn, Gould & Anderson (1963) proved that cadmium exerts its overall effect in the territory of distribution of the superior epididymal branch of the testicular artery, and by way of the latter itself. The fact that no damage occurs to the body or tail of the epididymis after administration of cadmium is confirmative. The action of the metals investigated here is most profound on the ductuli efferentes. Their effect on the head of the epididymis, and to a lesser extent on the body, would imply that if a vascular factor is involved it is not as specific as for cadmium. The regenerative processes of the duct system are much slower than those of the spermatogenic tissue, however, presumably owing to its normally lower mitotic rate.

It is also possible that another route of access of metal to the epididymal tissue exists. Since the spermatozoa very rapidly take up the metals in large amounts, it is feasible that some absorption of metal from the spermatozoa by the epithelial cells of epididymis may take place. This being the case it is logical to suppose that the ductuli efferentes would, therefore, absorb the greatest proportion of metal and so sustain the greatest damage.

The acute experiment produces a different effect on the head of the epididymis, namely a severe shrinkage of the tubules seen with cobalt and silver. This damage, which does not occur in the initial segment, has disappeared by the 4th day, as seen in Experiment 3. This is of interest in view of the special histochemical properties of this area (Nicander, 1957).

The epididymal spermatozoa show a great concentration of metal in them under dark ground microscopy, from the first day after injection. This metal content must, therefore, have been absorbed in some other way than by contribution from the Sertoli cells, since the time taken for passage of spermatozoa from the seminiferous tubules amounts to at least 4 days (Macmillan & Harrison, 1955).

Of the metals investigated here silver, nickel and cobalt (as aqueous salts) were investigated by Kamboj & Kar (1964) who administered a single subcutaneous injection to rats. They failed to notice any histological change in testis or epididymis by this route of administration at 2 or 7 days. This is not

in agreement with the results obtained here, since quite profound changes were obtained between 1 and 8 days.

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97. THE ABSORPTION AND EXCRETION OF 'MINOR' ELEMENTS BY MAN

2. COBALT, NICKEL, TIN AND MANGANESE

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In a previous paper Kent & McCance [1941] described the results of their work on the absorption and excretion of Ag, Au, Li, B, and V. The experiments now to be described have been carried out in a similar way. As before, the subjects have been either patients or normal persons and the same spectrochemical apparatus and analytical technique have been used. The previous article should be consulted for information about the analytical methods; a full description of the normal subjects and of the metabolic organization has been given by McCance & Widdowson [1941].

Cobalt

Bertrand [1926] and his co-workers showed that Ni and Co were present in all samples of arable soil collected from European countries. They also found these elements in plants and in many human and mammalian organs [Bertrand & Machebœuf, 1926; Bertrand & Mokragnatz, 1925]. Woldwill [1907] reported that neither Ni nor Co was absorbed from the gut, but this must have been due to faulty analysis, for absorption has been demonstrated by others [Mascherpa, 1927; Simesen, 1939]. The facility with which 'coast' disease in sheep can be cured by a Co drench [Askew & Dixon, 1936; Wunsch, 1937] and the ease with which polycythaemia can be produced by the oral administration of Co [Josland, 1939] are excellent proofs that at any rate small quantities must be absorbed. If Co has once been absorbed or has been given parenterally, the literature suggests that it is excreted partly by the kidney and partly by the intestine and that the route depends to a large extent upon the nature of the compound and upon how it has been administered. Untersteiner [1931], for example, found that divalent Co was more rapidly eliminated than trivalent Co. Simesen [1939] recovered in the urine of the next 24 hr. 80% of the Co which he had injected as $[Co_3CO(NH_3)_4]Cl$ subcutaneously into rabbits. This compound was excreted unchanged by the kidney. Mascherpa & Perito [1931], who administered $CoCl_2$ to guinea-pigs by the same route, recovered from the urine during the following 10 days less than half the quantity injected. Le Goff [1927] injected 21 mg. of $CoCl_2$ intramuscularly into a man and recovered 6·8 mg. of the salt in the urine within the next 18 hr. He recovered a much smaller quantity (2·41 mg.) from another patient who was a diabetic. Unfortunately the fæces were not examined.

The presence of Co in bile was demonstrated long ago by Stuart [1884]. This was not confirmed by Mascherpa [1927], but has been substantiated by Caujolle [1930] after intravenous administration of the chloride.

The subject of the present experiment was a male hospital patient suffering from carcinoma of the stomach. His kidneys were functioning normally and he

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did not vomit during the studies. Excluding the preliminary and after periods the experiment lasted 2 weeks. During the first, which served as the control, the patient was given a weighed diet, and urine and fæces were collected quantitatively. During the 2nd week the diet was repeated in every possible respect and urine and fæces were collected as before. On 5 of the 7 days of the 2nd week $CoCl_2$ was injected intravenously and in all 13 mg. of Co were administered in this way. The urine and fæces of both weeks were analysed for Co, but not the food. The results are given in Table 1. The 1st week's data show that the food

Table 1. *The metabolism of cobalt*

Week	Co injected intravenously mg.	Co excreted, mg.			Urine Co as % of total
		Urine	Fæces	Total	
1	0	0·24	1·01	1·25	17
2	13	2·30	1·78	4·14	57
Excess of 2 over 1	13	2·06	0·77	2·89	74

must have contained appreciable amounts of Co and that only 17 % of it passed through the kidney into the urine. During the week in which the injections were given there was a tenfold increase in the urinary excretion and a much smaller increase in the faecal excretion. Assuming the Co intake by mouth to have been the same in both weeks, the results show that of the 13 mg. which were injected intravenously 2·89 were excreted in the week, 74 % of this amount by the kidney. This single experiment confirms in broad outline the results of previous workers on animals and of Le Goff [1927] on man. It suggests that the gut is the main channel of excretion for the Co in natural foods, probably because relatively little is absorbed. Once Co has reached the tissues, however, it indicates that the processes of elimination are very slow, and that the kidney is the organ chiefly responsible. In the rate at which the Co was excreted the present results differ from those of Copp & Greenberg [1941], who administered radioactive Co to two rats and found that 90 % of the Co injected intraperitoneally into one animal was excreted within 4 days. This rapid elimination may be peculiar to the rat.

Nickel

The occurrence and distribution of Ni in soil, plants and in human and animal organs was studied by Bertrand and his fellow workers [Bertrand & Machebœuf, 1925; 1926; Bertrand, 1926]. They found that Ni and Co had similar distributions in nature, but that animals tended to contain more Co than Ni, plants and soils more Ni than Co [Bertrand & Machebœuf, 1926]. Muscherpa [1927] and others have studied the absorption and excretion of Ni and its salts. Large doses, whether by mouth or injection, produce a muco-haemorrhagic enteritis, and there is general agreement that this metal is excreted into the intestine rather than by the kidney. This is thought to explain why early workers found that Ni was not absorbed after being taken by mouth. It has been demonstrated in the bile by Stuart [1884], Lehmann [1909] and Canjolle [1937]. Few records have been found of experiments on man, and none of the elimination of Ni after intravenous or subcutaneous administration. A general account of the absorption, excretion and pharmacology of both Ni and Co has been given by Hendrych & Weden [1931].

Two normal men were the subjects for the present studies. Each received daily intravenous injections of $NiCl_2$ during the second week of a long metabolism experiment. The results are shown in Table 2, and it will be seen that before the

Table 2. *The absorption and excretion of nickel*

Subject	Length of period days	Ni intake mg./period: Injected	Ni intake mg./period: In food	Ni excretion mg./period: In urine	Ni excretion mg./period: In fæces	Balance mg.	Ni 'recovered' mg.
E. B.	7		2·25	1·57	0·73	− 0·04	
	7	0	1·99	3·42	0·69	+ 0·88	3·8
	7		2·67	3·16	1·19	− 1·68	
N. K.	7		—	2·20	1·20	—	
	7	20	5·51	6·70	1·33	+ 17·18	
	4		2·21	2·88	0·50	− 1·26	
	7		3·43	3·48	2·11	− 2·16	7·4
	7		3·20	2·61	2·05	− 1·40	

injections were made there was more Ni in the urine than in the fæces. This suggests a reasonably good absorption of the traces of Ni normally present in food. E. B. was almost exactly in balance at this time. N. K.'s balance cannot be given since the figure obtained for his food suggests that it became contaminated with Ni from cutlery during its preparation for analysis. Great precautions were subsequently taken to work only with wooden utensils. During the period of injection the output of Ni rose in the urine of both subjects, so that they eliminated more by this channel than they took in with their food, and they continued to do so in later periods. Clearly Ni was being excreted in the urine. The faecal outputs did not rise during the period of Ni injections, and in subsequent periods they varied in quantity but tended to be slightly higher than they had been during the preliminary week. Only 42 % of the Ni injected into E. B. was recovered and 37 % of that given to N. K. Ni, like Co and Sn (*vide infra*), was excreted slowly and rather incompletely in these experiments and the organ mainly concerned was the kidney, not the gut.

Tin

The metabolism and pharmacology of Sn have been studied sporadically since foods began to be preserved in cans. Most authors are in agreement that foods may become contaminated with Sn from tinned containers, but small quantities of the metal have also been found in fresh foodstuffs [Boyd & De, 1933; Bertrand & Curon, 1934]. Within recent years Sn salts have had a vogue in the treatment of furunculosis and most of the proprietary preparations are intended to be given subcutaneously. Apart, therefore, from the possibilities of industrial poisoning, it is evident that the body is frequently faced with the necessity of having to deal with small amounts of Sn salts. Buchmann & Schryver [1908], using human subjects, and Datta [1910], working with rats, came to the conclusion that tin was poorly absorbed and that small amounts taken by mouth were excreted mainly in the fæces. Sn given by subcutaneous or intravenous injection to animals has been reported to be rapidly removed from the circulation and to be excreted slowly and incompletely by the kidney [Engar & Badlander, 1887; Buchmann & Schryver, 1908; Salant *et al.* 1914; 1918; Salant, 1920]. No account has been found of the excretion of Sn after intravenous or subcutaneous administration to man.

Two normal men acted as subjects for the present investigations and each received a total of 28 mg. of Sn as 'Stanoxyl' by daily intravenous injection during the 2nd week of a 21-day metabolism experiment. The 1st week served as a control, during which the absorption and excretion of the Sn present in the food was followed. The results are given in Table 3. Both men were roughly in

Table 3. *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 faeces	Balance mg./period	Sn recovered mg.
E. H.	7	0	14·4	7·2	6·6	+ 0·6	
	7	28	13·1	12·6	7·3	+ 21·2	19·7
	7	0	12·1	13·1	11·0	− 12·0	
R. M.	7	0	12·8	8·8	2·9	+ 1·1	16·7
	14	28	30·5	36·6	10·6	+ 11·3	

balance during period I and were excreting 52 and 75% of their whole outputs of Sn in the urine. These figures are much higher than those of Datta [1940] or of Buchanan & Schryver [1908]. During subsequent periods the injected Sn was slowly excreted. 70% of the amount administered was recovered from E. H. and 60% from R. M. The kidney excreted most of this extra Sn, although the amount in the faeces also rose slightly. The part played by the kidney can be appreciated more fully if the amounts in the food and urine are compared before and after the injections were given.

Manganese

The regular occurrence of Mn in plants and animals—and hence in food—has been a well-established fact for many years [Bertrand, 1939], and many tables have been published showing the usual range of Mn concentration in various foodstuffs [Lindow & Peterson, 1927; Skinner & Peterson, 1928; Davidson, 1929; Remington & Shiver, 1930; Richards, 1930; Peterson & Skinner, 1931]. Perla *et al.* [1939] found that rats might retain little or none of the Mn naturally present in the food, practically all of it passing out in the faeces. When inorganic Mn was added to the food so that the intake was raised from 0·036 to 0·165 and later to 13·15 mg./rat/day, 25% of the dose was absorbed and retained. Skinner *et al.* [1931] obtained somewhat similar results, but found much higher percentages of Mn normally excreted in the urine.

The kidney has never been found to play an active part in the elimination of Mn [Harnack, 1901]. Normally, only a very small part of the Mn in the food is excreted by this organ, and the figure of 20%, which Skinner *et al.* [1931] obtained, seems very high in the light of other work. Mn taken by mouth may increase slightly the amount in the urine, but the proportion so excreted always falls [Perla *et al.* 1939; Skinner *et al.* 1931]. Reiman & Minot [1920] showed that these conclusions held good also for human subjects, but in their opinion part at least of the Mn in the faeces represents Mn that has been absorbed and subsequently eliminated. These workers found that after a man had taken 8 g. of franklinite, containing 0·77 g. of Mn, the blood Mn might rise from 0·012 to 0·024 mg./100 ml. within an hour, and that after patients with biliary fistulae had taken 5 g. of franklinite by mouth the Mn in the bile might rise to 10 times its previous level. They held therefore that at least a part of the Mn in the faeces represented metal which had been absorbed, only to be re-excreted. Few references have been found to experiments in which Mn was injected into animals and none to such experiments on man. Cahn [1884] injected toxic doses of Mn salts into rabbits, killed the animals shortly afterwards and analysed their organs. He concluded that Mn so administered was eliminated mainly by the intestine. Quite recently Greenberg & Campbell [1940] have used a radioactive

isotope, Mn^{54}, to follow the fate of Mn in the body. 1 mg. of Mn^{54} was injected intraperitoneally into 1 rat and during the subsequent 4 days 90·7 % of it was recovered in the fæces. The result would have been more convincing if the Mn had been given intravenously or subcutaneously rather than intraperitoneally. The quantity excreted in the urine was too small to have any significance and the remaining 9·3 % of the injected Mn^{54} was found in the bodily organs.

One woman and two men—all normal persons—were the subjects of the present experiments. Each received 4–7 injections of Mn butyrate during the 2nd of a 4-period metabolism experiment. E. B. received a diet designed to contain very little Mn; A. M. and P. S. took 40–50 % of their calories in the form of white flour. In other respects the latter's diets were freely chosen. A. M. (the woman), who received 14·3 mg. of Mn, and E. B., who received 19·8 mg., excreted little or none of the injected dose within the time of observation. P. S., however, who received 31·5 mg., probably excreted 16·1 mg. in the fæces during the last 3 weeks of his experiment, but the balances were somewhat irregular and unconvincing. The full results are to be found in Table 4.

Table 4. *The absorption and excretion of manganese*

Subject	Period no. and length	Mn intake mg./period: By mouth	Mn intake mg./period: Injected	Mn excretion mg./period: Urine	Mn excretion mg./period: Fæces	Balance mg./period	Mn recovered mg.
A. M.	1 (7 days)	15·5		0·7	13·7	+ 1·1	
	2 (7 days)	19·7	14·1	0·4	17·5	+ 10·1	
	3 (7 days)	23·1		0·3	24·9	− 1·8	3·1
	4 (7 days)	15·8		0·9	18·0	− 3·1	
E. B.	1 (6 days)	10·3		0·3	10·3	− 0·3	
	2 (6 days)	13·4	19·8	0·2	14·5	+ 18·5	
	3 (6 days)	12·5		0·1	12·0	+ 0·1	0
	4 (6 days)	14·0		0·1	8·2	+ 6·0	
P. S.	1 (7 days)	46·5		1·2	48·0	− 2·7	
	2 (7 days)	43·5	31·5	0·3	59·0	+ 15·7	
	3 (7 days)	32·1		0·1	37·6	− 5·4	10·1
	4 (7 days)	30·5		0·3	25·1	+ 5·1	

In no subject was there any evidence that any of the injected Mn was excreted in the urine. So far as the urine is concerned these results are essentially in agreement with those of most previous workers. It is evident, however, that Mn is not excreted freely, if at all, by the human bowel when it is injected in the amounts used for these experiments. The partial excretion shown by P. S. may have been the result of the larger doses he received, or of the smaller storage capacity which he possibly possessed. At any rate the difference is thought to have been genuinely one of metabolic behaviour and not to have arisen from contaminations or analytical inconsistencies. The Mn in the foods and fæces of P. S. and in some of the specimens of A. M. was determined chemically by the periodate method of Willard & Greathouse [1917]. The results agreed satisfactorily with those obtained spectrographically, and actually the figures given for P. S. in Table 4 were those obtained by the chemical method.

Table 4 shows that the Mn intakes may vary considerably on natural foods. One of the easiest ways of raising them is to eat a large amount of brown bread, for bran is very rich in Mn. 100 g. of 92 % flour were found to contain 2·15 mg. of Mn and 100 g. of 69 % flour only 0·49 mg. Table 5 shows the balances of 2 persons when they were deriving 40–50 % of their calories from 69 % flour and from 92 % flour.

Table 5. *Manganese intakes and excretions on diets containing large amounts of white and brown flour*

Subject	Diet	Mn intake in food mg./week	Mn excretion, mg./week Urine	Faeces	Total	Balance mg./week
A. M.	White flour	15·5	0·7	13·7	14·4	+1·1
	Brown flour	59·6	0·2	56·2	56·4	+3·2
E. W.	White flour	18·8	0·4	19·2	19·6	−0·8
	Brown flour	61·8	0·6	60·8	61·4	+0·4

Taking the results in Tables 4 and 5 together, they show that the urinary excretion of Mn, like that of Fe, is negligibly small, whatever the intake by mouth or injection. The fact that injecting Mn in these doses did not necessarily provoke any excretion of the metal by either kidney or gut recalls that the human animal has been shown to react to injections of Fe in exactly the same way [McCance & Widdowson, 1938], and illustrates the biochemical and pharmacological affinities of the two metals, some of which were pointed out long ago by Cahn [1881].

Summary

Metabolism experiments on men and women, combined with intravenous injections of Co, Ni, Sn and Mn salts, have shown that

(1) One man excreted about 20% of his food Co in the urine. Injected Co was excreted slowly, mainly by the kidney.

(2) Two men excreted 60–70% of their food Ni in the urine. Injected Ni was excreted slowly, mainly by the kidney.

(3) Two men excreted between 50 and 80% of their food Sn in the urine. Injected Sn was excreted very largely by the kidney.

(4) Only a very small part of the Mn in the food was excreted in the urine, and there was no increase after the intravenous injections of Mn salts. Two persons retained the whole of the injected Mn, a third excreted about 50% by the bowel.

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HISTOCHEMICAL OBSERVATIONS ON THE FIXATION AND ELIMINATION OF DERIVATIVES OF TIN THROUGH THE LIVER IN THE RAT AND THE RABBIT IN RELATION TO THE MODE OF ADMINISTRATION

By L. Khau-Van-Kien and Thai Tuong

We have been able to observe histochemical detection of tin introduced experimentally into the organism and phenomena of fixation and elimination at the level of the liver in the rat and in the rabbit intoxicated by mineral and organic derivatives of tin, soluble and insoluble, administered parenterally and orally, ending in intoxications that are acute, massive, subacute and chronic over a rather long period (six months).

The results of the localizations show first of all in the course of the cited cases of intoxication notable differences permitting their characterization, as well as of the products used, according to the place of localization of the tin in the hepatic region and also according to the histological and cytological changes observed.

In acute intoxication, the derivatives administered orally and parenterally are 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 more or less dilated, with the mobilization of the Kupffer cells, which fix or set the tin. The mineral or organic derivatives, recognizeable by their particular colorations,[1] are found at the beginning in the blood cells, in certain endothelial and parietal cells of the portal spaces and of the centrolobular veins; then there is produced an irritative reaction of the endothelial walls, with the partial destruction of the vascular walls, if the quantity of the derivatives of tin is greater. At this moment the granulations of tin penetrate more directly into the parenchymatous cells in the region of the vessels; but the quantity of tin in the cells of the hepatic parenchyma is much weaker than that in the vascular territory and in the cells of the reticuloendothelial system. The soluble organic products (di-iododiethyl tin) are found in this case in the form of granulations in a much larger quantity than the mineral derivatives (dissolved ~~tin~~ stannous chloride, stanoxyl, oxide of tin and chloride of tin in association). Organic products in acute intoxication cause only a few lesions in the hepatic parenchyma (death is rapid from nervous intoxication); on the other hand, the mineral derivatives are more irritating in regard to the vascular walls.

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1. L. Khau-Van-Kien and Thai Tuong, Annales d'Histochimie, 1956, v. 1, p. 15.

In subacute and chronic intoxications, which approximate therapeutic conditions, and in accidental intoxications, the derivatives of tin after having surcharged the Kupffer cells proceed to become fixed in the hepatic cells in a larger quantity. There would be a certain equalization, then a predominance of localization in the cells of the parenchyma at the level of the vessels and in the hepatic cells with a double nucleus; this fixation is accompanied by the elimination of the tin from the cell and from the liver by a process of dissolution of the granulations of tin fixed by the cell being indicated by a bright aureole around the granule or by refractive cytoplasmic vacuoles where the granulations have disappeared. It is then accompanied by a more definite attack on the hepatic cells by cytoplasmic infiltration of the granulations of tin with vacuolization or necrosis. In addition to the parenchyma of which the conjunctive spaces are more involved, the walls of the veins are thickened with the beginnings of sclerosis in the portal spaces and certain hepatic lobules. When the medication is very prolonged, the hepatic lesions are more marked with areas of diffuse, vacuolar, pigmented, and oily degeneracy. In the biliary portion of Glisson's capsule the tin is found on the conjunctive cells, histiocytes, and mastocytes. The organic derivatives, such as di-iododiethyl tin, in these cases cause hepatic lesions which are more marked with a greater fixation in the cell, causing degeneracies and necroses which are more extensive than those caused by mineral derivatives.

The results of our observations furthermore show that the penetration of the tin into the hepatic tissue is in accord with the way of administration and connected to the solubility of the tin derivatives.

The parenteral way permits the utilization of soluble mineral and organic derivatives of tin (dissolved ~~tin~~ stannous chloride, di-iododiethyl tin). These products introduced directly into the circulation avoid reabsorption by the intestinal barrier and remain in the course of acute intoxication preferably in the elements of the blood and vessel cells and are fixed by the reticuloendothelial system. They do not penetrate and are

Figure. The liver of the rat after subacute intoxication (Cl_2Sn) following reaction to the hematin for the detection of the tin (see page 2232 of text).

not fixed in the parenchyma which, if the defense system is penetrated, and this during a certain period, is the case in subacute and chronic intoxications where the greater fixation of tin produces degeneration and necroses, while the massive intoxication only causes the beginning of infiltration of the parenchyma around the vessels as we indicated above.

The oral way is more easily suited to the insoluble derivatives of tin, either mineral (oxide of tin, ~~tin~~ stannous chloride in powdered form, or in mixtures of the two[2]) or

2. Thai Tuong. Thèse Doct. Pharm., Montpellier, 1956.

organic (dibutylmalleate of tin which we have used with Gras[3]) mixed in food or administered orally in order to avoid loss of the products. By this method, localization of the tin in the parenchyma is greater in subacute and chronic intoxication. Although insoluble, these two kinds of derivatives are reabsorbed by the intestinal mucous membrane in appreciable quantity, however a little less than the dissolved ~~tin~~ stannous chloride. The tin is fixed chiefly by the Kupffer cells and by the parenchymatous cells near the portal vessels and the central lobular vessels. This way brings about a better impregnation of the parenchyma than the parenteral way. The mineral tin is fixed in the form of fine and numerous cytoplasmic granulations, while the organic tin yields coarser granulations which are more widespread in the lobule. The elimination of the tin fixed in the liver is well observed with chronic oral absorption. The product is eliminated by either the formation of a protein combination masking the tin or more probably the formation of an organosoluble compound carried away by the blood to be eliminated by the kidneys. The biliary canals of the portal spaces present only a very few granulations of tin in the course fo elimination. The cellular alteration and the necroses of the parenchyma by the products administered orally under these conditions appear more clearly and more cautiously with the organic derivatives.

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3. G.C. Gras, These Doct. Pharm., Montpellier, 1956.

Rats in cases of this kind of intoxication tolerate less well tin derivatives than do rabbits; the latter after a period of getting thinner adapt and get fatter again. This difference is due to the fact that the liver of the rabbit fixes tin derivatives intensively as is shown by the results given by Gras and the localizations given by our reactions with the same substance.

Our results consequently permit: (1) to define and realize in therapy against extraintestinal Platylhelminthes (bilharziasis from Schistosoma mansoni and distomatoses of the liver) the best conditions for impregnating to the greatest extent the infested regions and organs; (2) to improve the treatment of Platylhelminthes in general by tin salts while avoiding hepatic lesions; (3) to provide proof of the fixation and especially the elimination of the tin fixed in the liver by means of the blood and the kidneys rather than by the biliary way; (4) to show the existence of individual reactions in relation to the tin salts and the power of fixation and elimination of these products.

gac

SOCIÉTÉ DE BIOLOGIE DE MONTPELLIER

SÉANCE DU 17 DÉCEMBRE 1956.

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Présidence de M. A. Loubatières.

Observations histochimiques sur la fixation et l'élimination des dérivés de l'étain par le foie du Rat et du Lapin en rapport avec le mode d'administration.

par L. KHAU-VAN-KIEN et THAI TUONG.

Nous avons pu observer après détection histochimique de l'étain introduit expérimentalement dans l'organisme, des phénomènes de fixation et d'élimination au niveau du foie du Rat et du Lapin, intoxiqués par des dérivés minéraux, organiques, solubles et insolubles de l'étain, administrés par la voie parentérale et orale, aboutissant à des intoxications aiguë, massive, subaiguë et chronique pendant une assez longue période (six mois).

Les résultats de localisations montrent d'abord, au cours des cas d'intoxication cités, des différences notables permettant de les caractériser, ainsi que les produits utilisés, selon le lieu de la localisation de l'étain dans le territoire hépatique, et aussi selon les modifications histologiques et cytologiques observées.

Dans l'intoxication aiguë, les dérivés administrés par voie orale et parentérale se retrouvent dans le foie, dans les vaisseaux et dans les éléments du sang, très peu dans le parenchyme hépatique. L'étain congestionne le foie au niveau des vaisseaux et des capillaires plus ou moins dilatés, avec mobilisation des cellules de Kupffer qui fixent l'étain. Les dérivés minéraux ou organiques reconnaissables par leurs colorations particulières (1) se retrouvent au début dans les cellules sanguines, dans certaines cellules endothéliales et pariétales des vaisseaux des espaces portes et des veines centrolobulaires ; puis il se produit une réaction irritative des parois endothéliales avec destruction partielle des parois vasculaires, si la quantité des dérivés de l'étain est plus importante. A ce moment les granulations d'étain pénètrent plus directement dans les cellules parenchymateuses du voisinage des vaisseaux ; mais la quantité d'étain dans les cellules du parenchyme hépatique est bien plus faible que celle du territoire vasculaire et des cellules du système réticulo-endothélial. Les produits organiques solubles (di-iododiéthyl-étain) se retrouvent dans ce cas sous forme de granulations en quantité bien plus importante que les dérivés minéraux (chlorure stanneux dissous, stanoxyl, oxyde d'étain et chlorure d'étain associé). Les produits organiques dans l'intoxication aiguë provoquent peu de lésions dans le parenchyme hépatique (la mort est rapide par intoxication nerveuse) ; par contre les dérivés minéraux sont plus irritants pour les parois vasculaires.

Dans les intoxications subaiguës et chroniques plus proches des conditions thérapeutiques, et des intoxications accidentelles, les dérivés de l'étain après avoir surchargé les cellules de Kupffer, vont se fixer dans les cellules hépatiques en quantité plus importante, il y aurait une certaine égalisation, puis une prédominance de localisation dans les cellules du parenchyme au niveau des vaisseaux et dans les cellules hépatiques à double noyau ; cette fixation s'accompagne d'une élimination de l'étain hors de la cellule et du foie par un processus de dissolution des granulations d'étain fixé par la cellule, se traduisant par une auréole claire autour du granule, ou par des vacuoles cytoplasmiques réfringentes où les granulations ont disparu. Elle s'accompagne ensuite d'une atteinte plus accusée des cellules hépatiques par infiltration cytoplasmique de granulations d'étain, avec vacuolisation ou nécrose. A côté du parenchyme dont les travées conjonctives sont plus accusées, les parois des veines sont épaissies, avec des débuts de sclérose dans les espaces portes et certains lobules hépatiques. Lorsque la médication est très prolongée, les lésions hépatiques sont plus marquées avec des plages de dégénérescence diffuse, vacuolaire, pigmentaire et graisseuse. Dans la portion biliaire de la capsule de Glisson l'étain se retrouve sur les cellules conjonctives, histiocytes et mastocytes. Les dérivés organiques comme le di-iododiéthyl-étain dans ces cas donnent des lésions hépatiques

(1) L. Khau-Van-Kien et Thai Tuong, *Annales d'Histochimie*, 1956, t. 1, p. 15.

plus marquées, avec fixation plus importante dans la cellule, pro-quant des dégénérescences et des nécroses plus étendues que les dé-vés minéraux.

Les résultats de nos observations montrent en outre que la pé-tration de l'étain dans le tissu hépatique est en rapport avec la v d'administration et liée à la solubilité des dérivés staniés.

La voie parentérale permet d'utiliser les dérivés solubles mi et organiques de l'étain (chlorure stanneux dissous, di-iodo-di étain). Ces produits introduits directement dans la circulation tant la résorption par la barrière intestinale, restent au cours de l'i toxication aiguë de préférence dans les éléments du sang et les ce les des vaisseaux et sont fixées par le système réticulo-endothé

Foie du Rat après intoxication subaiguë (Cl$_2$Sn) après réaction à l'hémaléine pour la détection de l'étain.

Ils ne pénètrent et ne se fixent dans le parenchyme qui si le système de défense est débordé, et ceci pendant une certaine période, c'est le cas des intoxications subaiguës et chroniques où la fixation plus im-portante d'étain produit des dégénérescences et nécroses, alors que l'intoxication massive ne provoque qu'un début d'infiltration du pa-renchyme autour des vaisseaux comme nous l'avions signalé plus haut.

La voie orale s'accommode plus facilement des dérivés insolubles de l'étain, minéraux [oxyde d'étain, chlorure stanneux en poudre, ou association des deux (2)], organiques [dibutylmaléate d'étain que nous

(2*) Thai Tuong, Thèse Doct. Pharm., Montpellier, 1956.

avons utilisé avec Gras (3)], mêlés à l'alimentation, ou administrés *per os* pour éviter des pertes de produits. Par cette voie la localisation de l'étain dans le parenchyme est plus importante dans l'intoxication subaiguë et chronique. Bien qu'insolubles, ces deux sortes de dérivés sont résorbés par la muqueuse intestinale en quantité appréciable, cependant un peu moins que le chlorure stanneux dissous. L'étain est surtout fixé par les cellules de Kupffer et les cellules parenchymateuses proches des vaisseaux portes et centrolobulaires. Cette voie réalise une meilleure imprégnation du parenchyme que la voie parentérale. L'étain minéral est fixé sous forme de granulations cytoplasmiques fines et nombreuses, et l'étain organique donne des granulations plus grosses et plus répandues dans le lobule. L'élimination de l'étain fixé dans le foie s'observe bien avec l'absorption orale, chronique. Le produit est éliminé par formation, soit de combinaison protéinique masquant l'étain, soit plus vraisemblablement par formation de composé organosoluble entraîné par la voie sanguine pour être éliminé par les reins. Les canaux biliaires des espaces portes ne présentent que très peu de granulations d'étain en cours d'élimination. L'altération cellulaire et les nécroses du parenchyme par les produits administrés par la voie orale dans ces conditions, apparaissent plus nettement et plus précocement avec les dérivés organiques. Les rats dans ces intoxications supportent moins bien les dérivés staniés que les lapins, ces derniers après une période d'amaigrissement s'adaptent et grossissent. Cette différence est due à ce que le foie du Lapin fixe intensément les dérivés de l'étain, comme le montrent les résultats donnés par Gras et les localisations données par nos réactions avec la même substance.

Nos résultats ont pour conséquence de permettre : 1) de préciser et de réaliser dans la thérapeutique contre les Plathelminthes extra-intestinaux (bilharziose à *Schistosoma mansoni* et les diverses distomatoses hépatiques) les meilleures conditions pour imprégner au maximum les régions et organes infestés ; 2) d'améliorer le traitement des Plathelminthes en général, par les sels d'étain tout en évitant les lésions hépatiques ; 3) d'apporter une preuve de la fixation et surtout de l'élimination de l'étain fixé dans le foie, par la voie sanguine et rénale plus que par la voie biliaire ; 4) de démontrer l'existence de réactions individuelles vis-à-vis des sels d'étain en rapport avec le pouvoir de fixation et d'élimination de ces produits.

(3*) G. C. Gras, Thèse Doct. Pharm., Montpellier, 1960.

...st, January, 1969, Vol. 94, pp. 71–74 71

The Determination of Tin in Canned Foods with Quercetin

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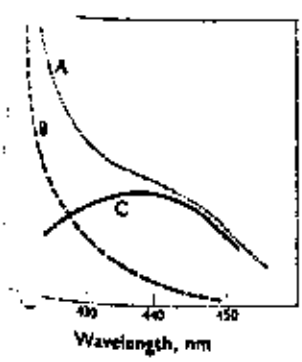
A colorimetric method is described for determining tin in canned foods by means of the orange-coloured complex it forms with quercetin. Interference from iron(III) is masked by thiourea. The method gives results comparable with those obtained by the dithiol method but manipulation is easier, and it is, therefore, more suitable for routine analytical determinations, particularly when the tin content is relatively high.

...higher trace levels found in canned foods, tin has been determined by gravimetric ...metric methods,[1] and by several colorimetric procedures based on dithiol.[2] Morin (...,2',4'-pentahydroxyflavone) was introduced by Feigl[3] as a spot-test reagent for tin. ...ovic and White[4] used flavonol (3-hydroxyflavone) for the fluorimetric determination ...of tin. Grimaldi and White,[5] in determining traces of zirconium colorimetrically ...quercetin (3,5,7,3',4'-pentahydroxyflavone), noticed the large interference caused by ...d suggested its possible use for the determination of that metal. Liška[6] described a ...d in which quercetin was used for the determination of small amounts of tin in copper ... Lyaskovskaya and Krasil'nikova[7] adapted Liška's method to the determination of ...ood, and later, in a paper on canned meat,[8] compared the quercetin and dithiol methods. We have critically examined the applicability of Lyaskovskaya and Krasil'nikova's ...d to canned foods and, with some modification, have found it to be suitable for the ...10 to 500 p.p.m. of tin. The final technique evolved is described below.

...s AFFECTING THE TIN – QUERCETIN COMPLEX DEVELOPMENT—

...ercetin is a yellow solid which, when added in ethanolic solution to an acidified ...of tin(IV), produces a bright yellow – orange colour. Fig. 1 shows the absorption ...of the complex and of quercetin. The tin complex has a maximum absorbance ...nm, and this wavelength was used for all subsequent absorption readings. Various ...affecting the optimum conditions for the complex formation were studied.



...1. Absorption spectra of A, ...quercetin; B, quercetin; ...plex

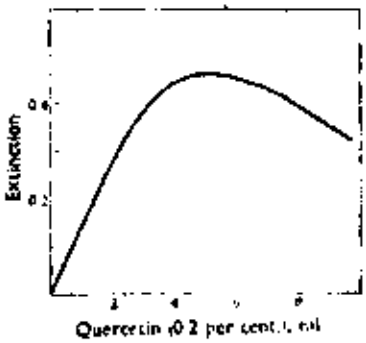


Fig. 2. Effect of quercetin concentration

...ercetin and its coloured tin complex are soluble in 50 per cent. aqueous ethanol, but ...in will not remain dissolved in more dilute ethanol. The effect of quercetin concen... in 50 ml of solution containing tin is indicated in Fig. 2. Between 4 and 6 ml of a

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0·2 per cent. solution of quercetin in ethanol gave optimum colour response, correspond in the absence of tin, to an extinction of just under 0·4 in a 4-cm cell. As quercetin is unsta in alkaline solution, the acidic, wet-oxidised sample solutions require careful neutralisa before adjustment to standard acidity. This was achieved by Karvánek, Čurda and by controlling the amount of acid used in the digestion process, so that neutralisation avoided. The final volume of the concentrated sample digestion solution was adjust 5·0 ml with concentrated sulphuric acid; this requires considerable experimental sim neutralisation technique described here, however, has proved both satisfactory and rela simple. Sodium carbonate solution was found to be the most satisfactory alkali for neutr tion, giving more consistent results than ammonia solution; sodium hydroxide also erratic results, thus indicating possible localised hydrolysis effects. 2,4-Dinitrophen suitable indicator for this purpose, as it is colourless below pH 2 and yellow at a high The result of varying the amount of hydrochloric acid added to the neutralised solut illustrated in Fig. 3, which shows that between 4 and 6 ml of 2·5 N hydrochloric ac necessary for optimum colour development in 50 ml of solution. Neutralised sol allowed to stand for 20 minutes before being re-acidified gave significantly lower re

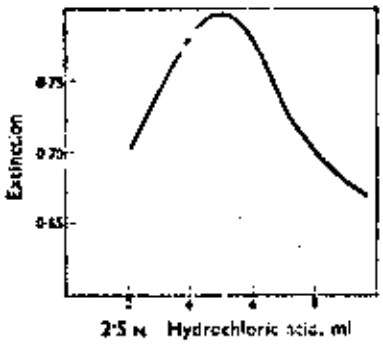

Fig. 3. Effect of acidity

The colour-complex development was substantially complete after 15 minut no further change after 30 minutes. Solutions of both quercetin and its tin comp found to be stable, a fall of only 0·02 extinction units in a 4-cm cell occurring after 3 After 5 days, the quercetin stock solution gave a slightly lower reagent blank reading test, but had suffered no loss of sensitivity.

A calibration graph, prepared under the optimum conditions, obeyed Beer's la the useful range of 0 to 50 μg of tin ($E1_{cm}$ = 2250 at 437 nm). For the test, the le tin is kept within this range by taking 2·0 ml aliquots from 50-ml dilutions of the d samples. Larger aliquots cause opalescence in the colour development because of the ha level of inorganic matter in the ethanolic mixture.

PREPARATION OF SAMPLE SOLUTION—

To avoid the risk of insoluble tin formation by dry ashing, samples of canned food prepared for analysis by wet oxidation with nitric and sulphuric acids. When silica are used with a Meker-type burner to increase the rate of oxidation, a 5-g sample can be digested in 15 minutes.

METHOD

REAGENTS—

Nitric acid, sp. gr. 1·42 Analytical-reagent grade.
Sulphuric acid, sp. gr. 1·84 Analytical-reagent grade.
2,4-Dinitrophenol solution, 0·1 *per cent. in 50 per cent. aqueous ethanol.*
Sodium carbonate solution, 10 *per cent. w/v, aqueous.*
Hydrochloric acid, 2·5 N.
Thiourea, saturated aqueous solution.
Quercetin, 0·2 *per cent. w/v in ethanol.*
Ethanol, 95 *per cent.*

PROCEDURE—

Transfer a known weight of the comminuted sample, containing not more than 1000 μg of tin, to a silica digestion flask. Add 10 ml of concentrated nitric acid and, after 10 minutes, add 5 ml of concentrated sulphuric acid. Boil the mixture vigorously and, immediately charring commences, add small amounts of concentrated nitric acid. Continue the oxidation [illegible] manner until charring ceases and then boil until white fumes of sulphur trioxide appear. [illegible] the mixture. (If silica flasks are used this is effected instantaneously by immersion in [illegible] water.) Add 20 ml of water and transfer the oxidised solution quantitatively to a [illegible] calibrated flask. Make up to the mark with water and mix. By pipette, introduce [illegible] ml of this solution into another 50-ml calibrated flask and add 0·2 ml of 2,4-dinitrophenol [illegible]. Add 10 per cent. sodium carbonate solution, dropwise with continual agitation of [illegible] flask, until the first appearance of a yellow colour. Discharge this colour by dropwise addition of 2·5 N hydrochloric acid and immediately add a further 5·0 ml of acid. Add 3 ml of thiourea solution and 5·0 ml of quercetin reagent, followed by 25 ml of ethanol; dilute to [illegible] with water and mix. After 30 minutes, measure the extinction in a 4-cm cell at 437 nm against a reagent blank solution produced in the same manner as the sample; 2-cm cells are [illegible] if the tin level is high. From a calibration graph determine the amount of tin in [illegible] sample weight taken.

CONSTRUCTION OF THE CALIBRATION GRAPH—

Prepare a standard tin solution by dissolving 0·0500 g of pure tin in 50 ml of boiling concentrated sulphuric acid. Cool and cautiously add this solution to 120 ml of water, with [illegible] cooling, and transfer to a 200-ml calibrated flask; make up to the mark with 25 per cent. sulphuric acid (1 ml of solution = 250 μg of tin). To a series of digestion flasks containing 5 ml of concentrated sulphuric acid and 10 ml of concentrated nitric acid, add 0, 1·0, [illegible] 3·0 and 4·0 ml of the standard tin solution. Boil until the nitric acid is expelled and [illegible] fumes appear. Cool, add 20 ml of water and proceed with the colour development [illegible] as described above. Construct the calibration graph relating extinction to micro[illegible] of tin.

DISCUSSION AND RESULTS

Recoveries of tin added to samples of baked beans in tomato sauce are given in Table I. [illegible] recoveries of tin were obtained when the level of tin per flask exceeded 1000 μg. This [illegible] to be caused by condensation of tin, under the influence of nitric acid, to meta[illegible] acid. Some opalescence is often apparent when more than 2000 μg of tin are present, [illegible] it is advisable to reject digestion solutions that are not clear and bright and to use a [illegible] sample for oxidation.

TABLE I

RECOVERY OF TIN ADDED TO SAMPLES OF BAKED BEANS

Weight of [illegible] taken,	Tin in sample weight taken, μg	Tin added, μg	Total tin found, μg	Added tin recovered, μg	Percentage recovery
[illegible]	575	500	1035	460	92
[illegible]	575	1250	1660	1085	87
[illegible]	575	2500	2400	1825	73
2·0	230	500	740	510	102
2·0	230	750	970	740	99
[illegible]	290	500	750	460	92
[illegible]	290	1000	1175	885	89
2·0	116	125	240	124	99
2·0	116	250	350	234	93
2·0	116	500	590	480	96
2·0	116	750	850	740	99

The effect of substances in wet-ashed food, which might interfere, was examined. Iron [illegible] level corresponding to gross contamination of food (10,000 p.p.m.) is effectively masked [illegible] the addition of thiourea. No difficulty was experienced with samples containing up to [illegible] p.p.m. of copper when the pH conditions indicated are adhered to. The normal concentrations of sulphate and nitrate ions resulting from wet oxidation show no effect on the tin

Lang, N. A., ed., 1952
Lange's Handbook of Chemistry, 8th Edition
Handbook Publishers, Inc., Sandusky, Ohio

Comparative Studies on the Reabsorption of Heavy Metals in Vivo and in Vitro

by

G. Leopold, E. Furukawa, W. Forth and W. Rummel.

A mechanism takes part in the absorption of Fe when it is floated across the mucosa, which is characterized by metabolic dependence (1,4) and high specificity (3). The system responsible for this is localized chiefly in the jejunum [lit. in (2)]. It seemed to us worthwhile to establish whether this transport system might not in certain circumstances also be used by other heavy metals. A comparison of the jejunum and the ileum with regard to the penetration and binding of heavy metals in tissues served to clarify this question. The experiments were carried out in vitro on small everted intestinal sacs and on tied intestinal loops of the rat in situ.

Fe and its next related Co differ from all other metals in that their absorption by the jejunum is several times higher than by the ileum. In the in vitro experiments this also applies to Mn and Sb (see Table). Cr is the only metal of which the ileum in vitro absorbs several times the quantity absorbed by the jejunum. Accordingly the ileum also binds more Cr. However, this difference cannot be statistically confirmed in vivo (see Table).

Tl is marked by the highest rate of absorption. In vivo it is twice that of Co, which is in second place. The great

difference between the jejunum and ileum characteristic of Fe and Co is absent; the difference is significant, but small (see Table). This also applies to the quantity bound in the intestinal wall of the two segments. If we take the more marked absorption of a heavy metal by the jejunum as an indication of the fact that the system responsible for Fe absorption is used in a decisive manner for transport across the mucosa, it can then be established that the toxicologically important metals Hg, Tl, Sn, Pb and Sb above all, but also the biometals Cr, Cu and Zn are not utilized in this floating-out mechanism.

Retention of the metals was determined by measurement of the radioactivity of the animals between the 3rd and 17th day after the administration through a stomach tube. The metals were administered in a concentration of 5.10^{-6} M/l (in 2 ml 0.9% NaCl solution, pH 2), with the exception of Pb, the concentration of which was 5.10^{-5} M/l. The following sequence was found, in which Fe lies behind Sn at the beginning with a 2% decrease of its content in the animals between the 3rd and 17th day after administration and Tl lies at the end with a decrease of 92%.

Sn > Fe > Sb > Zn > Hg > Pb > Co > Mn > Cr > Tl.

Table. Absorption of biometals or toxicologically important heavy metals from tied intestinal loops in situ in rats. Length of intestinal segment: 20 cm; jejunum (I): from the flexura duodenojej. onwards; ileum (II): from the valvula ielocecalis onwards. Filling: 2 ml sodium chloride solution, pH 2. Metal concentration: 5.10^{-6}M/l. This concentration in vitro (everted sac preparation) had no influence on the ascending transport of glucose. Duration of experiment: 1 hour. The figures are the means of 5-15 experiments; they give the absorbed quantity of metal in % of supply ± the standard deviation s_x. The absorbed quantity of metal was established by determination of the radioactivity of the animals in a small-animal installation. 1. Metals. 2. Supplied as.

Tabelle. Resorption von Biometallen bzw. toxikologisch wichtigen Schwermetallen aus abgebundenen Dünndarmschlingen in situ bei Ratten. Durchlänge: 20 cm; Jejunum (I): von der Flexura duodenojej. an; Ileum (II): von der Valvula ileocaecalis an. Füllung: 2 ml Kochsalzlösung, pH 2. Metallkonzentration: $5 \cdot 10^{-6}$ M/l. Diese Konzentration blieb in vitro (everted sac-Präparat) ohne Einfluß auf den Bergauftransport von Glucose. Versuchsdauer: 1 Std. Die Zahlen sind die Mittelwerte aus 5 – 15 Versuchen; sie geben die resorbierte Metallmenge in Prozent des Angebots ± der Standardabweichung s_x wieder. Die resorbierte Metallmenge wurde durch Bestimmung der Radioaktivität der Tiere in einer Kleintieranlage ermittelt

1. Metalle	Cr		Mn		Fe		Co	
2. Angebot als	^{51}Cr-$CrCl_3$		^{54}Mn-$MnCl_2$		^{59}Fe-$FeCl_3$		^{60}Co-$CoCl_2$	
	I	II	I	II	I	II	I	II
	5,1	7,3	1,8	1,0*	6,2	0,7*	32,9	2,6*
	± 1,7	± 4,5	± 0,7	± 0,3	± 2,3	± 0,5	± 15,1	± 1,1

Metalle	Cu		Zn		Hg		Tl	
Angebot als	^{64}Cu-$CuSO_4$		^{65}Zn-$ZnCl_2$		^{203}Hg-$HgCl_2$		^{204}Tl-Tl_2SO_4	
	I	II	I	II	I	II	I	II
	14,9	11,4	6,0	8,1	0,7	0,5	81,1	61,4*
	± 11,5	± 4,3	± 3,0	± 3,7	± 0,2	± 0,1	± 6,7	± 7,0

Metalle	Sn		Pb		Sb	
Angebot als	^{113}Sn-$SnCl_2$		^{203}Pb-Pb-Acetat		^{125}Sb-$SbCl_3$	
	I	II	I	II	I	II
	0,5	0,1	6,9	6,2	7,4	2,3*
	± 0,3	± 0,2	± 1,9	± 2,0	± 2,6	± 4,8

* = $p < 0{,}05$ gegen I.
against

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Vergleichende Untersuchung der Resorption von Schwermetallen in vivo und in vitro

Comparative Studies of the Absorption of Heavy Metals in Vivo and in Vitro

Von G. Leopold, E. Furukawa, W. Forth und W. Rummel

Bei der Resorption von Fe ist an der Durchschleusung durch die Mucosa ein Mechanismus beteiligt, der sich durch metabolische Abhängigkeit [1,4] und durch hohe Spezifität [3] auszeichnet. Das hierfür verantwortliche System ist vor allem im Jejunum lokalisiert (Lit. s. bei [2]). Es erschien von Interesse zu prüfen, ob dieses Transportsystem unter Umständen auch von anderen Schwermetallen mitbenutzt wird. Der Klärung dieser Frage diente der Vergleich von *Jejunum* und *Ileum* hinsichtlich Durchtritt und Bindung der Schwermetalle im Gewebe. Die Versuche wurden mit umgestülpten Darmsäckchen in vitro (everted sac) und an abgebundenen Darmschlingen in situ bei Ratten durchgeführt.

Fe und sein nächster Verwandter Co unterscheiden sich von allen übrigen Metallen dadurch, daß ihre Resorption durch das Jejunum mehrfach höher ist als durch das Ileum. Bei den in vitro-Versuchen gilt das auch für Mn und Sb (vgl. Tabelle). Cr ist das einzige Metall, von dem in vitro durch das Ileum ein Vielfaches im Vergleich zum Jejunum resorbiert wird. Dementsprechend bindet das Ileum auch mehr Cr. In vivo ist dieser Unterschied statistisch jedoch nicht zu sichern (vgl. Tabelle).

Tl fällt durch die höchste Resorptionsrate auf. Sie ist in vivo mehr als doppelt so hoch wie die von Co, das an zweiter Stelle steht. Der für Fe und Co kennzeichnende große Unterschied zwischen Jejunum und Ileum fehlt; der Unterschied ist zwar signifikant, aber klein (vgl. Tabelle). Das trifft auch für die in der Darmwand der beiden Segmente gebundene Menge zu. Nimmt man die bevorzugte Resorption eines Schwermetalls durch das Jejunum als Anzeichen dafür, daß das für die Fe-Resorption verantwortliche System beim Durchtritt durch die Mucosa maßgebend mitbenutzt wird, dann ist festzustellen, daß vor allem die toxikologisch wichtigen Metalle Hg, Tl, Sn, Pb und Sb, aber auch die Biometalle Cr, Cu und Zn diesen Durchschleusungsmechanismus nicht in Anspruch nehmen.

Die *Retention* der Metalle wurde durch Messung der Radioaktivität der Tiere zwischen dem 3. und 17. Tag nach Verabfolgung mit der Schlundsonde bestimmt. Die Metalle wurden in einer Konzentration von $5 \cdot 10^{-6}$ M/l (in 2 vol 0,9% NaCl-Lösung, pH 2) verabreicht, eine Ausnahme bildet Pb, dessen Konzentration $5 \cdot 10^{-5}$ M/l betrug. Dabei ergab sich folgende Reihe, bei der am Anfang hinter Sn das Fe mit einer Abnahme des Gehaltes der Tiere zwischen dem 3. und 17. Tag nach der

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Vergleichende Untersuchung der Resorption von Schwermetallen in vivo und in vitro

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Applikation um 2% und am Ende Tl mit einer Abnahme um 92% steht: Sn > Fe > Sb > Zn > Hg > Pb > Co > Mn > Cr > Tl.

Tabelle. Resorption von Biometallen bzw. toxikologisch wichtigen Schwermetallen aus abgebundenen Dünndarmschlingen in situ bei Ratten. Darmlänge: 20 cm; Jejunum (I): von der Fixierung duodenoileal an; Ileum (II): von der Valvula ileocaecalis an. Füllung: 2 ml Korkmilchlösung, pH 2. Metallkonzentration: $5 \cdot 10^{-5}$ M/l. Diese Konzentration blieb in vitro (vertical sac-Präparat) ohne Einfluß auf den Bergundransport von Glucose. Versuchsdauer: 1 Std. Die Zahlen sind die Mittelwerte aus 5–15 Versuchen; sie geben die resorbierte Metallmenge in Prozent des Angebots ± der Standardabweichung s_x wieder. Die resorbierte Metallmenge wurde durch Bestimmung der Radioaktivität der Tiere in einer Kleintierzentrifuge ermittelt

Metalle	Cr		Mn		Fe		Co	
Angebot als	^{51}Cr-$CrCl_3$		^{54}Mn-$MnCl_2$		^{59}Fe-$FeCl_3$		^{60}Co-$CoCl_2$	
	I	II	I	II	I	II	I	II
	5,1	7,3	1,8	1,0*	5,2	0,7*	32,0	2,6*
	± 1,7	± 4,5	± 0,7	± 0,3	± 2,3	± 0,5	± 15,1	± 1,1

Metalle	Cu		Zn		Hg		Tl	
Angebot als	^{64}Cu-$CuSO_4$		^{65}Zn-$ZnCl_2$		^{203}Hg-$HgCl_2$		^{204}Tl-Tl_2SO_4	
	I	II	I	II	I	II	I	II
	11,9	11,4	6,9	8,1	0,7	0,5	81,1	61,1*
	± 11,5	± 4,2	± 3,0	± 3,7	± 0,2	± 0,1	± 0,7	± 7,0

Metalle	Sn		Pb		Sb	
Angebot als	^{113}Sn-$SnCl_2$		^{203}Pb-Pb-Acetat		^{122}Sb-$SbCl_3$	
	I	II	I	II	I	II
	0,5	0,1	6,9	6,2	7,4	2,3*
	± 0,3	± 0,2	± 1,9	± 2,0*	± 2,0	± 4,8

* = $p < 0{,}05$ gegen I.

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THE CACOTHELINE METHOD OF DETERMINING TIN IN FOOD PRODUCTS

By I.V. Medvedeva

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The present standard method for determining tin in food products was developed on the basis of Obe's iodometric method; it has significant defects and has been criticized repeatedly (M.P. Bolotov, 1936; A.G. Bosin and S.E. Spektor, 1938; N.D. Podobed and F.A. Chigirinskaya, 1938; Ye.N. Sergeyeva, 1936). The weakest place in this procedure is the use of the wet combustion method, requiring the outlays of a great deal of time, reagents, and accompanying exudation of a tremendous amount of harmful vapors and gases. Furthermore, the results of such analysis are not precise or easily reproducible and subject to significant fluctuation.

The errors of the method depend on many reasons: titration with unstable solutions of iodine and hyposulfate, inclusion in test sample of a large amount of proteins and fats, the presence of iron and copper responsible for large-size errors on the high side (80 percent and higher according to Ye.N. Sergeyeva). Among the defects of the method, there should also be included in our opinion the significant effect exerted by the temperature and pH on the results of titration.

Our proposed method of quantitative determination of tin in food products is based on the color reaction of ions of bivalent tin with cacotheline, nitro derived brucine of undetermined struction. Some authors (F.P. Tredvell and V.T. Goll, 1946; F. Fayql', 1933) attach to it an empirical formula $C_{20}H_{22}N_2O_5(NO_2)_2$, others (A.S. Komerovskiy, 1950) -- $C_{21}H_{21}(OH)_2(NO)_2N_2O_3 \cdot HNO_3$. The mechanism of reaction has not yet been investigated.

The aqueous solution of cacotheline in acidic solutions of bivalent tine a violet coloration evidently due to the color of the product of cacotheline reduction.

Data in the literature and experimental verification of the sensitivity and specificity of certain qualitative reactions to tine have shown that the reaction of bivalent tin to cacotheline is most appropriate for the development of a quantitative method based on it.

On the basis of this reaction, we (I.I. Paul', V.A. Vinogradova, I.V. Medvedeva, 1957) developed in 1954 a micromethod for the determination of tin in the air of industrial buildings. In the present study, we have for our objective completion of the method so that it can be applied to new conditions stemming from dry calcination of food products. This made it necessary for us to develop conditions for burning organic substances and eliminating losses of tin and speeding up incineration, to determine the specificity, sensitivity, and precision of the reaction of the tin to cacotheline under the conditions of the method, to elucidate the effect of

glazed crucibles containing SnO_2 on the disclosed amount of tin, and finally to provide a comparative characterization of the standard and the new (cacotheline) methods.

These questions were resolved as the result of numerous experiments.

Taking into account, the deficiencies of the wet method of burning, we finally decided on the method of dry calcination in a muffle. We selected experimentally an accelerator additive, determined its concentration for binding the tin into a nonvolatile compound and accelerating the burning. Burning with magnesium acetate was found to be most effective, 2 ml of a 10 percent solution which completely binds the tin added to a food product. The samples burn rapidly without the formation of fused sediments; 32 control experiments involving burning of different food products with additions of accelerators showed that magnesium acetate (10 percent) does not act on the glaze of the crucible.

To elucidate the specificity of the method, 7 series of experiments were conducted involving burning of food products with the addition of 20 different elements and magnesium acetate. It was established in this connection that elements usually present in canned goods (iron, calcium, phosphorus, copper, and others) did not interfere in the determination of the tin under the conditions of the proposed method.

Relative Determination of Tin in Food Products

Минерализованные продукты (1)	(2) Найдено олова (в мг)					
	(3) при стандартном методе			(5) при какотелиновом методе		
	(4) с добавлением			(6) с добавлением		
	Sn 5 мг (7)	Sn 5 мг, Fe 5 мг (8)	Sn 5 мг, Cu 5 мг (9)	Sn 5 мг (10)	Sn 5 мг, Fe 5 мг (11)	Sn 5 мг, Cu 5 мг (12)
Хлеб воздушно-сухой (13)	4,88	4,5	9,8	5,02	5,0	4,78
Мясо воздушно-сухое (14)	4,85	5,2	14,1	4,9	4,67	4,40
Молоко козье . . . . (15)	4,49	4,31	9,44	4,43	4,91	4,50
Вермишель с маслом . (16)	4,76	5,17	8,8	4,79	5,0	4,55
Капуста . . . . . (17)	5,4	5,3	8,6	5,02	4,9	4,06
Желатин . . . . . (18)	4,85	4,8	10,5	4,98	5,0	4,95
Томат . . . . . (19)	3,78	4,61	—	4,95	4,93	4,85
Рацион воздушно-сухой . (20)	4,44	4,3	7,7	4,65	5,02	4,93
(21) В среднем . . .	4,68	4,79	9,85	4,81	5,05	4,70
Ошибка (22)	−6,4%	−4,2%	+97%	−3,2%	+1%	−6%

1. Name of product
2. Tin found (in mg)
3. With standard method
4. With addition of
5. With cacotheline method
6. With addition of
7. Sn, 5 mg
8. Sn, 5 mg; Fe, 5 mg
9. Sn, 5 mg; Cu, 5 mg
10. Sn, 5 mg
11. Sn, 5 mg; Fe, 5 mg
12. Sn, 5 mg; Cu, 5 mg
13. Bread, air-dried
14. Meat, air-dried
15. Goat's milk
16. Vermicelli with butter
17. Cabbage
18. Gelatine
19. Tomato
20. Ration, air-dried
21. Average
22. Error

The new method is distinguished by high sensitivity and accuracy, good reproductibility; it provides perfectly satisfactory results beginning with 0.005 mg of tin, with an error not exceeding ±10 percent. The high sensitivity permits making a significant decrease in the weight of the portion, resulting in large savings of time and reagents.

Checking of the method against standard solutions of tin added to food products and in analyses of canned goods produced coinciding results (see table).

Principle of the method. A weighted portion is calcinated in the presence of magnesium acetate by means of the dry method; the ash is diluted in muriatic acid; the tin is determined through titration with a cacotheline solution.

Essential reagents: (1) muriatic acid with a specific gravity of 1.19; (2) muriatic acid 1:1; (3) nitric acid 33 percent; (4) nitric acid 6 percent; (5) brucine; (6) alcohol; (7) ether; (8) chemically pure granulated zinc; (9) zinc shavings (best results are produced with shavings from a turning lathe using granulated zinc fused without mixing to avoid affecting the granular structure, which makes it possible to get short bits of shavings); (10) chemically pure metallic tin; (11) standard solution of tin (0.05 g of chemically pure tin cut with scissors in the form of fine acicular shavings, which is diluted in 10 ml of concentrated muriatic acid in a small conic flask equipped with a Bunsen valve for heating in a water bath; the obtained solution is transferred into a 50-ml measuring flask and caused to reach the mark with a bidistillate; 1 ml of such a solution contains 1 mg of tin; when needed such a solution can be used to provide more diluted working solutions); (12) cacotheline prepared according to the method of A.M. Kul'berg (1 g brucine is diluted in the cold in 5 ml of 33-percent nitric acid and then heated for 15 minutes in a water bath with a temperature of 50-60°C; the red reactive solution gradually becomes yellow and precipitates yellow-red cacotheline crystals; after 4

hours, the sediment is drawn off and first washed in 6-percent nitric acid and then in acetone or alcohol until all acid is removed, and then finally with ether); (13) the titrated solution of cacotheline (0.153 g of cacotheline diluted in 200 ml of bidistillate in the cold; the amount of cacotheline in 1 ml of such a solution corresponds to 0.05 mg of tin; the exact titer of the solution is determined according to the standard solution of tin containing 0.1 mg of tin in 1 ml; for this purpose, 0.10-0.25 ml of standard solution is restored immediately prior to titration with 0.30-0.35 g of zinc shavings and then subjected to titration as described below in the course of analysis of the test samples; the titer for cacotheline is determined by taking the average for several titrations; the cacotheline solution is stable, when stored its titer does not change); (14) 10-percent solution of magnesium acetate.

Preparation of material for analysis. The test sample of the food is carefully mixed and pulverized in a porcelain mortar (homogeneity of the test sample is especially important in the case of a small portion). Weighted samples in the amount of 3 to 10 g are put in porcelain crucibles or cups preferably with a smooth surface. A 10-percent solution of magnesium acetate in the amount of 2-3 ml is added and carefully mixed with a glass blade, washing it with water later in the crucible. The test samples are then dried in a drying closet, carbonized on a plate, and placed in the muffle till

the ash is white or tinted with salts. Too intense a heat should be avoided in view of coking and the formation of coal as well as fusing of the precipitate with the glazed surface of the cup or crucible.

Dilution, reduction, and titration. The ash is diluted in 5 ml of muriatic acid 1:1 by heating in a water bath. The contents are transferred quantitatively into a graduated test tube or cylinder, washing the crucible or cup with water. After cooling, the volume of the sample is measured and carefully mixed; if the solution is murky, it is centrifuged. If the solution has a sediment, the tin is first determined in the clear part and then in the sediment. To determine the tin in the solution, about 0.30-0.35 g of zinc shavings are poured into the centrifuging test tube, from 0.1 to 2.0 ml of the test sample (depending on the anticipated concentration of the tin) are poured with a pipette and about 1 ml of muriatic acid with a specific gravity of 1.19. The test tube is corked with a rubber cork with a Bunsen valve to prevent oxidation and to permit the shavings to react with the muriatic acid.

The reaction should not proceed too strongly; in such a case, the shaving floats to the top and the tin is not completely reduced. Immediately after the zinc is diluted, the sample is titrated with a solution of cacotheline from Shilov's microburet. The first drop of cacotheline in the presence of bivalent tine brings about the appearance of a

pale violet coloration. With further titration, intensity increases and the coloring changes to violet and then brown. Titration is continued until the brown-violet coloring changes to brown-yellow. In titration, one should use such amounts of the test sample which would have not more than 20 γ of tine (better 10-15 γ); it is easier to establish transition in color with such a concentration. If the concentration of tin in the test sample is large, the sample should be diluted and an aliquot part used for the titration.

Since the change of coloring occurs gradually rather than abruptly, in mastering the method, one should carefully look for changes in coloring at the time of titration of a standard solution.

If the test sample contains tin in the sediment, the sediment is washed with water, centrifuged, and the water is then poured off by turning the test tube; the operation is repeated 2-3 times.

There is added to the sediment in the test tube 1 ml of concentrated muriatic acid and 1 granule of zinc. A stormy reaction occurs in diluting the ainc. If there is tin in the sediment, it is separated during such energetic reduction in the form of black flakes of tin sponge, which are dissolved in 0.5-1.0 ml of concentrated muriatic acid by heating in a water bath. After the dissolving of the sponge and cooling, the volume is measured and the solution is mixed; then the tin is determined in the same way as in the fluid part of the test sample. The amount of detected tin in the sediment

is added to the amount of the tin found in the solution.

The results of the analysis are computed according to the formula

$$X = \frac{0.05 \cdot A \cdot V \cdot K}{V_1}$$

where X is the amount of tin in the test sample in mg; A -- the amount of cacotheline going into the titration; 0.05 -- the amount of tin in mg corresponding to 1 ml of cacotheline; V -- the volume of the entire test sample; V_1 -- the volume of the test sample taken for titration; K -- the coefficient of correction which is determined by dividing the volume of theoretical cacotheline (1 ml of cacotheline corresponds to 0.05 mg of tin) by the volume of cacotheline actually going into the titration of the 0.05 mg of tin.

Example. An analyzed test sample has a volume of 7 ml. There is used 0.2 ml of the test sample for the titration with 0.37 ml actually going into titration; K = 1. Then

$$X = \frac{0.05 \cdot 0.37 \cdot 7.1}{0.2} = 0.65 \text{ mg of tin in the test sample.}$$

CONCLUSIONS

1. A new method has been developed to determine the amount of tin in food products by means of titration with cacotheline.

2. A method is proposed for dry calcination of food products with the addition of a 10-percent solution of magnesium acetate which eliminates losses of tin and speeds up the calcining process.

3. Metal ions usually found in food products do not interfere with the determination of the tin.

4. The method is accurate and sensitive and gives perfectly satisfactory results beginning with 0.005 mg of tin, with error not exceeding ±10 percent. Its high sensitivity permits making the weighed sample smaller, thereby reducing the time spent in making the analysis. Analysis of portions of canned goods in 10 or more cans may be carried out in the course of one work day.

5. The method has undoubted advantages over the standard method: it is considerably more simple and can be performed more quickly, accurately, and sensitively, more specifically and cheaply, while completely eliminating the danger of wet calcination.

6. In verifying standard solutions of tin added to food products and in analyzing canned goods, the method has given results that coincide.

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КАКОТЕЛИНОВЫЙ МЕТОД ОПРЕДЕЛЕНИЯ ОЛОВА В ПИЩЕВЫХ ПРОДУКТАХ

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Существующая стандартная методика определения олова в пищевых продуктах, разработанная на основе йодометрического метода Obe, имеет существенные недостатки и неоднократно подвергалась критике (М. П. Болотов, 1936; А. Г. Босин и С. Е. Спектор, 1938; Н. Д. Подобед и Ф. А. Чигиринская, 1938; Е. Н. Сергеева, 1936). Наиболее слабым местом методики является применение мокрого способа сжигания, требующего большой затраты времени, реактивов и сопровождающегося выделением огромного количества вредных паров и газов. Результаты анализа при этом не отличаются точностью, хорошей воспроизводимостью и подвержены значительным колебаниям.

Погрешности метода зависят от многих причин: от титрования [illegible] стойкими растворами йода и гипосульфита, содержания в пробе большого количества белков и жиров, присутствия железа и меди, дающих большую ошибку в сторону завышения (по Е. Н. Сергеевой [illegible] и выше). К недостаткам метода следует, по нашему мнению, отнести также значительное влияние на результаты титрования pH и температуры.

Предлагаемый нами метод количественного определения олова в пищевых продуктах основан на цветной реакции ионов двухвалентного олова с какотелином, нитропроизводным пруниной неустановленного строения. Одни авторы (Ф. П. Трехвеля и В. Т. [illegible], 1936; Ф. Ф[illegible], 1937) приписывают ему эмпирическую формулу $C_{14}H_{22}N_4O_4(NO_2)$ [illegible]

гие (А. С. Комеровский, 1950) — $C_{24}H_{24}(OH)_2(NO_2)N_2O_6 \cdot HNO_3$. Механизм реакции еще не исследован.

Водный раствор какотелина вызывает в кислых растворах двухвалентного олова фиолетовое окрашивание, обусловливаемое, по-видимому, окраской продукта восстановления какотелина.

Литературные данные и экспериментальная проверка чувствительности и специфичности известных качественных реакций на олово показали, что реакция двухвалентного олова с какотелином является наиболее подходящей для разработки на ее основе количественного метода.

В 1954 г. нами (И. И. Пауль, В. А. Виноградова, И. В. Медведев, 1957) на основе этой реакции был разработан микрометод определения олова в воздухе производственных помещений. В настоящей работе мы поставили целью доработать метод применительно к новым условиям, получаемым в результате сухого озоления пищевых продуктов. Для этого нам необходимо было разработать условия сжигания органических веществ, устраняющие потери олова и ускоряющие сжигание, установить специфичность, чувствительность и точность реакции олова с какотелином в условиях метода, выяснить влияние на открываемое количество олова глазури тиглей, содержащей SnO_2, и, наконец, дать сравнительную характеристику стандартного и нового (какотелинового) методов.

В результате многочисленных экспериментов эти вопросы были решены.

Учитывая недостатки мокрого способа сжигания, мы остановились на способе сухого озоления в муфеле. Экспериментальным путем была подобрана добавка ускорителя, установлена концентрация его, требуемая для связывания олова в нелетучее соединение и ускорения сжигания. Наиболее эффективным оказалось сжигание с ацетатом магния, 2 мл 10% раствора которого полностью связывают олово, добавленное в пищевой продукт. Пробы сгорают быстро, без образования сплавленных осадков; 32 контрольных опыта сжигания различных пищевых продуктов с добавками ускорителей показали, что ацетат магния (10%) не действует на глазурь тигля.

Для выяснения специфичности метода были проведены 7 серий опытов сжигания пищевых продуктов с добавлением 20 различных элементов и ацетата магния. При этом установлено, что обычно присутствующие в консервах элементы (железо, кальций, фосфор, медь и др.) в условиях предлагаемого метода определению олова не мешают.

Сравнительное определение олова в пищевых продуктах

Наименование продукта	Найдено олова (в мг)					
	при стандартном методе			при какотелиновом методе		
	с добавлением			с добавлением		
	Sn 5 мг	Sn 5 мг, Fe 5 мг	Sn 5 мг, Cu 5 мг	Sn 5 мг	Sn 5 мг, Fe 5 мг	Sn 5 мг, Cu 5 мг
Хлеб воздушно-сухой	4,88	4,5	9,8	5,02	5,0	4,7[illegible]
Мясо воздушно-сухое	4,85	5,2	14,1	4,9[illegible]	4,67	4,4[illegible]
Молоко козье	4,49	4,31	9,44	4,43	4,91	4,50
Вермишель с маслом	4,76	5,17	8,8	4,79	5,0	4,5[illegible]
Капуста	5,4	5,3	8,6	5,02	4,9	4,66
Желатин	4,85	4,8	10,5	4,98	5,0	4,95
Томат	3,78	4,61	—	4,9[illegible]	4,93	4,[illegible]
Рацион воздушно-сухой	4,44	4,3	7,7	4,[illegible]	5,02	4,9[illegible]
В среднем	4,68	4,79	9,85	4,84	5,05	[illegible]
Ошибка	−6,4%	−4,2%	+97%	−3,2%	[illegible]1%	[illegible]

Новый метод отличается высокой чувствительностью и точностью, хорошей воспроизводимостью и дает вполне приемлемые результаты, начиная с 0,005 мг олова, с ошибкой, не превышающей ± 10%. Высокая чувствительность позволяет значительно уменьшить навеску, что дает большую экономию времени и реактивов.

Проверка метода на стандартных растворах олова, добавленных в пищевые продукты, и на анализах консервов дала совпадающие результаты (см. таблицу).

Принцип метода. Навеска сплавляется сухим путем в присутствии ацетата магния; зола растворяется в соляной кислоте; олово определяется путем титрования раствором какотелина.

Необходимые реактивы: 1) соляная кислота удельного веса 1,19; 2) соляная кислота 1 : 1; 3) азотная кислота 33%; 4) азотная кислота 6%; 5) бруцин; 6) спирт; 7) эфир; 8) цинк гранулированный химически чистый; 9) цинковая стружка (лучшие результаты дает стружка, приготовленная на токарном станке из гранулированного цинка, сплавленного без перемешивания во избежание нарушения зернистой структуры, способствующей получению короткой стружки-крошки); 10) олово металлическое химически чистое; 11) стандартный раствор олова (0,05 г химически чистого олова, нарезанного ножницами в виде тонкой игольчатой стружки, растворяют в 10 мл концентрированной соляной кислоты в небольшой конической колбе, снабженной клапаном Бунзена, при нагревании на водяной бане; полученный раствор переносят в мерную колбу на 50 мл и доводят до метки бидистиллятом; 1 мл такого раствора содержит 1 мг олова; из такого раствора по мере надобности приготовляют более разбавленные рабочие растворы); 12) какотелин, приготовленный по методу А. М. Кульберга (1 г бруцина растворяют на холоду в 5 мл 33% азотной кислоты и 15 минут нагревают на водяной бане при 50—60°; постепенно красный реакционный раствор желтеет и выпадают желто-красные кристаллы какотелина; через 4 часа осадок отсасывают и промывают сначала 6% азотной кислотой, затем ацетоном или спиртом до полного удаления кислоты и под конец эфиром); 13) титрованный раствор какотелина (0,153 г какотелина растворяют в 200 мл бидистиллята на холоду, содержание какотелина в 1 мл такого раствора соответствует 0,05 мг олова; точный титр раствора устанавливают по стандартному раствору олова, содержащего 0,1 мг олова в 1 мл; для этого 0,10—0,25 мл стандартного раствора непосредственно перед титрованием восстанавливают 0,3—0,35 г цинковой стружки и титруют, как описано ниже в ходе анализа проб; титр какотелина рассчитывают, беря среднее из нескольких титрований; раствор какотелина устойчив, титр его при хранении не изменяется); 14) 10% раствор ацетата магния.

Подготовка материала к анализу. Пробу пищевого продукта тщательно перемешивают и растирают в фарфоровой ступке (однородность пробы особенно важна при малой навеске). Навески в количестве от 3 до 10 г берут в фарфоровые тигли или чашки, желательно с гладкой поверхностью. Добавляют 2—3 мл 10% раствора ацетата магния, тщательно перемешивают стеклянной лопаткой, смывая ее потом водой в тигель. Затем пробы высушивают в сушильном шкафу, обугливают на плитке и прокаливают в муфеле до белой или окрашенной солями золы. Сильного накала следует избегать ввиду коксования образующегося угля и сплавления осадка с глазурью чашки или тигля.

Растворение, восстановление и титрование. Золу растворяют в 5 мл соляной кислоты 1 : 1 при нагревании на водяной бане. Содержимое количественно переносят в градуированную пробирку или цилиндр, смывая тигель или чашку водой. После охлаждения замеряют объем пробы и тщательно перемешивают; если раствор мутный, то центрифугируют. Если в растворе имеется осадок, то олово определяют вначале в прозрачной части, а затем в осадке. Для определения олова в растворе в центрифужную пробирку насыпают около 0,30—0,35 г цинковой стружки, наливают пипеткой от 0,1 до 2 мл пробы (в зависимости от ожидаемой концентрации олова) и около 1 мл соляной кислоты удельного веса 1,19. Пробирку закрывают резиновой пробкой с клапаном Бунзена для предохранения от окисления и дают стружке реагировать с соляной кислотой.

Реакция не должна идти слишком бурно; в этом случае стружка всплывает и олово не успевает полностью восстановиться. Немедленно по окончании растворения цинка пробу титруют раствором какотелина из микробюретки Шилова. От первой капли какотелина в присутствии двухвалентного олова появляется бледно-фиолетовое окрашивание. При дальнейшем титровании интенсивность возрастает, окраска становится фиолетовой, затем бурой. Титрование производят до изменения буро-фиолетового окрашивания в буро-желтое. Для титрования следует брать такие количества пробы, в которых было бы не более 20 γ олова (лучше 10—15 γ); при такой концентрации легче установить переход окраски. Если концентрация олова в пробе велика, нужно разбавить пробу и на титрование брать аликвотную ее часть.

Поскольку переход окраски происходит постепенно, не резко, то при освоении метода надо тщательно присматриваться к переходу окраски во время титрования стандартного раствора.

Если проба содержит в осадке олово, то осадок промывают водой, центрифугируют, затем воду быстро сливают перемешиванием пробирки, операцию повторяют 2—3 раза.

К осадку в пробирке прилирают 1 мл концентрированной соляной кислоты и добавляют 1 гранулу цинка. Начинается бурная реакция растворения цинка. Если в осадке имеется олово, то при таком энергичном восстановлении оно выделяется в виде черных хлопьев оловянной губки, которую растворяют в 0,5—1 мл концентрированной соляной кислоты при нагревании на водяной бане. После растворения губки и охлаждения замеряют объем и перемешивают раствор, затем определяют олово так же, как в жидкой части пробы. Количество обнаруженного олова в осадке прибавляют к количеству олова, обнаруженного в растворе.

Результаты анализа рассчитывают по формуле

$$X = \frac{0{,}05 \cdot A \cdot V \cdot K}{V_1},$$

где X — количество олова в пробе в мг; A — количество какотелина, пошедшего на титрование; 0,05 — количество мг олова, соответствующее 1 мл какотелина; V — объем всей пробы; V_1 — объем пробы, взятой на титрование; K — коэффициент поправки, который устанавливается путем деления объема какотелина теоретического (1 мл какотелина соответствует 0,05 мг олова) на объем какотелина, фактически пошедшего на титрование тех же 0,05 мг олова.

Пример расчета. Анализируемая проба имеет объем 7 мл. На титрование взято 0,2 мл пробы, пошло на титрование 0,37 мл; $K = 1$. Тогда $X = \frac{0{,}05 \cdot 0{,}37 \cdot 7{,}1}{0{,}2} = 0{,}65$ мг олова в пробе.

Выводы

1. Разработан новый метод определения олова в пищевых продуктах посредством титрования какотелином.

2. Предложен способ сухого сжигания пищевых продуктов с добавлением 10% раствора ацетата магния, устраняющий потери олова и ускоряющий процесс сжигания.

3. Ионы металлов, обычно встречающиеся в пищевых продуктах, определению олова не мешают.

4. Метод точен, чувствителен и дает вполне приемлемые результаты, начиная с 0,005 мг олова, с ошибкой, не превышающей ±10%. Высокая чувствительность позволяет сократить навеску, а следовательно, и время на производство анализа. Анализ партии консервов в 10 и более банок можно произвести в течение одного рабочего дня.

5. Метод обладает бесспорными преимуществами перед стандартным методом: он значительно проще и быстрее по исполнению, точнее и чувствительнее, специфичнее и дешевле, полностью устраняет вредности мокрого сжигания.

6. При проверке на стандартных растворах олова, добавленных в пищевые продукты, и на анализе консервов метод дал совпадающие результаты.

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A CACOTHELINE METHOD OF DETERMINING TIN IN FOOD

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Summary

The paper describes a new titrometric method of quantitative determination of tin in foodstuffs, based on the color reaction of ions of bivalent tin with cacothelin. The incineration with a 10% solution of magnesium acetate was employed. The method ...

vesi[illegible] [illegible] to for[illegible] of canned food, produ[illegible] [illegible] of the [illegible] and the [illegible]ndard methods reve[illegible] the adva[illegible] of the [illegible] method: it is more specific, [illegible]ise, censsitive, as well as harmless and simple.

ON THE INCINERATION METHOD IN DETERMINING TIN IN FOODSTUFFS

by

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In the determination of tin, as of any other metal, in foodstuffs it is necessary to break down organic substances with which the tin is adsorptively or chemically bonded.

All methods for breaking down organic substances are basically reduced to various modifications of "dry" and "wet" incineration (S. M. Popov, 1931; A. A. Smirnova, 1934; I. M. Smorodintsev, A. N. Adova, 1934; M. P. Bolotov, 1936; A. G. Bosin, S. Ye. Spektov, 1938; I. D. Gadaskina, 1939; V. V. Tikhomirov, F. P. Shalaykin, 1941; A. V. Stepanov, 1947; Ye. B. Sendèl, 1949; M. N. Arkhangelov, 1950; Methods in Determining Microelements, 1950). Some authors (M. P. Bolotov, 1936; A. G. Bosin, S. Ye. Spektor, 1938) totally reject wet incineration in determining tin, since for an extremely long time it has required a great number of reagents and is accompanied by the liberation of toxic vapors and gases without eliminating, however, metal losses.

Large quantities of protein and fats can cause noticeable fluctuations in determination results (M. P. Bolotov, 1936). The opinion exists (B. Ya. Glazman, S. S. Barsutskaya, 1928; N. D. Podobed, F. A. Chigirinskaya, 1938) that some acidic methods of incineration, e.g. H_2SO_4 with addition of Berthollet's salt are unsuitable for breaking down organic metal compounds of tin or copper, while incineration with nitric acid, moreover, does not break down fats.

Dry incineration, in spite of its advantages (simplicity, inexpensiveness, absence of noxious vapors and gases, etc.) for a long

while did not find acceptance owing to significant losses (evaporation, transition into insoluble state) in direct incineration and the duration of incineration without the addition of accelerants (M. P. Bolotov, 1936; A. G. Bosin, S. Ye. Spektor, 1938). In direct dry incineration of canned products, M. P. Bolotov (1936) obtained a yield of tin from 4.4 to 96.1%. The author made the assumption that during incineration, a large role should be played by ash elements of the product being studied or various salts added to the substance being incinerated, which can eliminate losses of tin and accelerate the incineration process.

Various researchers proposed the addition of salts which, by accelerating the incineration process, simultaneously bind the tin into a non-volatile compound. Thus, Klein (1903) proposed magnesium oxide for accelerating the incineration of meat, blood, milk, and other products. His method was used by A. A. Smirnova (1934) for incinerating organic products containing arsenic and by M. P. Bolotov (1936) and A. G. Bosin (1938) for determining tin. The composition of the accelerant was supplemented by other substances: sodium chloride and acetic acid (M. P. Bolotov), sodium potassium acetate, ammonium chloride, and sodium chloride (A. G. Bosin).

The primary part of these accelerants is magnesium, which inhibits the evaporation of tin by apparently forming a compound akin to magnesium stannate.

Also described in the literature is alkaline incineration, i.e., incineration with the addition of sodium and potassium hydroxide in determining copper, boron, zinc, iron, and other elements in foodstuffs and biological materials (R. I. Lirtsman, L. M. Kul'berg, 1936); T. Ye. Gulyayeva, 1950; L. M. Kul'berg, P. A. Soyfer, 1951; Hoffman, Schweitzer, Dabby, 1940).

In determining tin in various canned products by the standard method using wet incineration, we produced significant variations in the amounts of tin discovered, especially in analyzing canned

products of animal origin, containing much fat and protein. For instance, in two parallel tests of canned "Cod Liver" we found 64.25 and 25 mg/kg of tin, "Broiled Cod" - 15.4 and 43.5 mg/kg, "Pork Stew" - 46.25 and 79.25 mg/kg, etc. Tests of these canned products burn extremely slowly and require a large amount of nitric acid and liberate much nitric oxide, incineration being accompanied by strong frothing.

The amount of sulfuric acid recommended by the All-Union Standard is insufficient for complete break down of organic substances in similar tests.

Thus, the wet method of incineration for determining tin in foodstuffs was found incomplete and therefore, during the development of a new method for determining tin in foodstuffs, we checked on the dry method of incineration in a muffle furnace.

At first, we conducted two series of tests on incinerating foodstuffs without adding accelerants. In the first series, products of vegetable and animal origin were analyzed which were free from tin, having added measured quantitites of the latter in the form of a standard solution of $SnCl_4$. In the second series of tests, tin was determined in samples of various canned products.

It was established that losses of tin produced during direct dry incineration in a muffle furnace are extremely appreciable and fluctuate within a wide range.

Thus, for instance, losses in incineration of air-dried bread with added tin are significantly higher (91%) than in incinerating air-dried nutritive ration (from 35 to 51%).

Losses in incinerating samples of canned vegetable products fluctuate from 2.7 to 77.6%, all the tin being in soluble form. In canned products of animal origin, tin losses are significantly less - down to 40.5%; on the average, they constitute 10.3%, but

most of the tin was found in the sediment.

In samples of canned fish products, apparently owing to their content of a large amount of calcium salts inhibiting the evaporation of tin, it was frequently totally discovered (even added in the form of a standard solution of $SnCl_4$), all the tin being found in the sediment (1,300γ; 1,166γ); while in solution there were either traces of it or it was totally absent (0; 18; 56γ).

The method developed by us earlier (1957) for converting insoluble tin compounds into solution by means of vigorous reduction of sediment with granular zinc in a H_2SO_4 medium allows us to totally reveal all the tin in the sediment.

According to Ye. B. Sendèl (1949), tin in wet and dry forms of incineration turns into β-metastannic acid, insoluble in acids. However, analyses conducted by us on various canned products showed that in incineration by the dry method, tin often can be revealed in solution. Specially run experiments (30 determinations) established that insoluble tin compounds (SnO_2) during incineration partially turn into soluble compounds.

Our experiments in incinerating foodstuffs without addition of accelerants showed that although in some cases we also totally discovered the tin (mainly in the sediment), more often it evaporated; moreover, incineration takes place slowly and incompletely, with the formation of molten ash.

Standing before us was the problem of finding an additive for dry incineration which would eliminate tin losses and accelerate incineration, as well as explain the effect on the tin quantity found of the enamel of the procelain crucible which contains SnO_2.

We tested alkaline incineration (with additive 0.1n NaOH) and incineration with magnesium acetate additive.

Table 1

Incineration of Foodstuffs with Addition of Tin and Magnesium Acetate

Name of Foodstuff	Weight, g	Added		Found	% error
		magnesium acetate	tin, γ	tin, γ	
bread, air-dr.	5	1ml 5%	500	366	-26.8
"	5	1ml 10%	500	370	-26.0
"	5	" "	500	380	-22.0
"	5	" "	200	163	-18.5
"	2	" "	200	190	-5.0
ration, a.-d.	5	4ml 1%	300	260	-13.4
bread, a.-d..	5	2ml 10%	1,000	1,000	0
"	5	" "	500	483.3	-3.3
"	5	" "	500	500	0
"	5	" "	500	500	0
"	5	3ml 10%	500	525	+5.0
"	5	2ml 10%	200	196	-2.0
"	10	" "	200	203	+1.5
"	10	" "	200	201.7	+0.85
meat, raw....	5	1ml 10%	500	455	-9.0
"	5	" "	500	355	-29.0
"	5	2ml 10%	500	511.7	+2.3
"	5	" "	500	505	+1.0
"	4	" "	100	101.5	+1.5
meat, rabbit, air-dried...	5	" "	200	195.5	-2.25
milk	10	" "	200	196.3	-1.83
vermicelli with oil...	10	" "	200	191.6	-4.2
cabbage.....	10	3ml 10%	500	501.5	+0.3
gelatine.....	2	2ml 10%	200	199.3	-0.35
tomato paste.....	10	" "	1,000	990.3	-0.97

An attempt at using alkaline incineration turned out unsuccessful, since findings were obtained either exaggerated or too low; incineration occurred slowly and was accompanied by swelling of the substance, formation of a relatively insoluble alloy, and breakdown of enamel of the porcelain and quartz vessel in which incineration took place.

An addition of magnesium acetate was more effective. To establish the optimum concentration of magnesium acetate, amounts of foodstuffs of vegetable and animal origin free from tin were mixed with a measured quantity of the latter from 100 to 1,000γ

Table 2

Quantitative Determination of Tin in Foodstuffs
(burnt with 2-3ml 10% solution of magnesium acetate)

name of foodstuff (canned products)	weight, g	Amount of tin, γ		Tin found, γ	% error	Incineration conditions
		found in weight	added			
sugar maize	10	201	--	204	+1.4	porcelain crucible
"	10	201	100	209.6	-0.4	quartz cruc.
"	10	302	---	305.4	+0.09	porcelain cr.
"	10	302	100	414	+3.9	quartz cr.
mandarin compote..	20	800	200	1010.8	+1.2	porcel. cruc.
"	20	800	---	808.5	+1.0	"
apricot compote...	10	1,038	---	1,037	-0.1	"
"	10	1,038	200	1,248	+0.96	"
pork stew..	10	205.0	---	203	-0.9	"
"	10	205.0	200	394	-2.7	"
"	10	205.0	200	409.5	+1.74	"
condensed milk.....	5	727	---	765	+5.2	"
"	5	727	200	931	+0.5	"
flounder in tomato pt.	10	3,025	---	3,157	+4.0	porc. dish
"	10	3,025	200	3,363.3	+4.2	quartz cruc.
"	10	1,042	---	1,060	+1.7	porc. cruc.
"	10	1,042	200	1,316	+7.1	quartz cruc.
chastik fish in tom.pt.	10	1,225	200	1,449.5	+2.0	porc. cruc.
"	10	1,225	---	1,178	-3.8	"
eel-pout in tom.paste.	10	1,472	---	1,498	+1.7	"
"	10	1,472	200	1,604.5	-4.5	"
flounder in oil	5	425.35	---	431.3	+1.4	"
"	5	425.35	200	627.7	+0.3	"
Average...					2.3%	

Findings produced upon adding 2-3ml 10% solution of magnesium acetate are fully acceptable; samples burn rapidly without losses, with the formation of crumbly and porous ash.

Analysis results of 23 samples of canned products (Table 2) also attest that the addition of 2-3ml 10% solution of magnesium acetate is fully satisfactory for binding tin; samples burn rapidly.

Error in determination does not exceed ±10%.

In order to eliminate possible effect of the enamel of a porcelain vessel on the tin determination results, incineration of all canned product samples having different additives was conducted simultaneously in a porcelain and quartz vessel. From Table 2 we can see that similar results are obtained in this connection.

Conclusions

1. Our research shows that the wet incineration method of foodstuffs in determining tin can be successfully replaced by dry incineration in the presence of magnesium acetate, which is an accelerant and tin-binding substance.

2. Without adding magnesium acetate, dry incineration occurs frequently slowly and incompletely, with large tin losses.

3. Analysis of 23 jars of potted products of vegetable and animal origin show that the method developed by us for dry incineration in the presence of 2-3ml 10% solution of magnesium acetate produces totally acceptable results. Samples burn rapidly, with the formation of a crumbly and porous ash. Error in determining tin equals ±2.3% and does not exceed ±7.1% in individual instances.

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The elaboration of a new method of quantitative test for tin in food products was preceded by a number of methods of wet any dry incineration.

These experiments proved the wet incineration, usually employed for determination of tin in foodstuffs, to be highly imperfect. Dry incineration with no admixtures or with the addition of some alkali often proceeds with losses of tin, is slow and incomplete. Besides, the addition of alkali tends to destroy the crucible enamel and to lixiviate the tin contained in it.

Dry incineration with the addition to the test sample of 2—3 ml. of a 10% solution of magnium acetate proceeds rapidly without any losses or destruction of crucible glaze and may be recommended for disintegration of organic substances during tests for tin.

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К МЕТОДИКЕ СЖИГАНИЯ ПРИ ОПРЕДЕЛЕНИИ ОЛОВА В ПИЩЕВЫХ ПРОДУКТАХ

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При определении наличия олова, как и любого другого металла, в пищевых продуктах необходимо разрушить органические вещества, с которыми олово адсорбтивно или химически связано.

В основном все методы разрушения органических веществ сводится к различным модификациям «сухого» и «мокрого» озоления (С. М. Новов, 1931; А. А. Смирнова, 1934; Н. М. Смородинцев, А. Н. Адова, 19..; М. П. Болотов, 1936; А. Г. Бесин, С. Е. Спектор, 1938; Н. Д. Галаскин, 1939; В. В. Тихомиров, Ф. Н. Шалайкин, 1941; А. В. Степанов, 19.. Е. Б. Сендэл, 1949; М. Н. Архангелов, 1950; Методы определения мик. элементов, 1950). Некоторые авторы (М. П. Болотов, 1936; А. Г. Бесин, С. Е. Спектор, 1938) при определении олова полностью отвергают мокрое озоление, поскольку оно весьма длительно, требует множества реактивов и сопровождается выделением вредных паров и газов, не устраняет, однако, потерь металла.

Большие количества белков и жира могут вызвать заметные колебания в результатах определения (М. П. Болотов, 1936). Имеется мнение (Б. Я. Глазман, С. С. Барсукова, 1928; Н. Д. Подобед, Ф. А. Чигирская, 1938), что некоторые кислотные способы сжигания, например серной кислотой с добавлением бертолетовой соли, непригодны для разрушения металлоорганических соединений олова или меди, а сжигание азотной кислотой, кроме того, не разрушает и жира.

Сухое сжигание, несмотря на время, шелка (простота, дешевизна, отсутствие вредных паров и газов и т. д.), долгое время не находило признания из-за значительных потерь летучивание, переход в нерастворимое состояние при прямом озолении и длительности сжигания без добавок ускорителей (М. П. Болотов, 1936; А. Г. Бесин, С. Е. Спектор, 1938). При прямом сухом сжигании в тиглях М. П. Болотов (1936) получил выход олова от 4,1 до 36,1%. Автор сделал предположение, что в процессе сжигания большую роль должны играть зольные элементы исследуемого продукта или различные соли, добавляемые к сжигаемому веществу, которые могут устранить потери олова и форсировать процесс сжигания.

Ранними исследователями было предложено добавление солей, которое, ускоряя процесс озоления, одновременно связывает олово в нелетучее соединение. Так, Klein (1903) предложил окись магния для ускорения сжигания мяса, крови, молока и других продуктов. Его метод использовала А. А. Смирнова (1934) для сжигания органических, содержащих металлы, веществ, а также М. П. Болотовым (1936) и А. Г. Бесиным (1938) для определения олова. Состав ускорителя пополняется другими веществами: хлористым натрием и уксусной кислотой (М. П. Болотов), уксуснокислой солью, хлористым аммонием и натрием (А. Г. Бесин).

Основной из этих ускорителей является магний, который, по-видимому, образует, по-видимому, соединение типа стеарата магния.

В литературе описывается также щелочное озоление, т. е. сжигание с добавлением гидроксидов натрия и калия при определении меди, бора, цинка, железа и других элементов в пищевых продуктах и биологических материалах (Р. Н. Лаременко, Л. М. Кульберг, 1936; Т. Е. Гусева, 1938; Л. М. Кульберг, Н. А. Сойфер, 1951; Hoffman, Schweigert, Bacon, 1944).

При определении олова в различных консервах стандартным методом с применением мокрого сжигания мы получали значительные колебания количеств обнаруживаемого олова, особенно при анализе консервов животного происхождения, содержащих много жира и белков. Например, в трех параллельных пробах консервов «Печени трески» было найдено 64,25 и 25 мг/кг олова, «Трески жареной» — 15,4 и 13,5 мг/кг, «Свиной тушенки» — 46,25 и 79,25 мг/кг и т. д. Пробы таких консервов сгорают или медленно, требуется большое количество азотной кислоты и выделяется много окислов азота, причем сжигание сопровождается сильным вспениванием.

Количества серной кислоты, рекомендуемого по ОСТ, недостаточно для полного разрушения органических веществ подобных проб.

Таким образом, мокрый способ сжигания при определении олова в пищевых продуктах оказался несовершенным, поэтому при разработке нового метода определения олова в пищевых продуктах мы остановились на сухом способе сжигания в муфеле.

Вначале нами проведены две серии опытов определения олова в пищевых продуктов без добавления ускорителей. В первой серии опытов использовались продукты растительного и животного происхождения, не содержащие олова, с добавлением дозированными количествами последнего в виде стандартного раствора $SnCl_2$. Во второй серии опытов определяли олово в пробах различных консервов.

При этом было установлено, что потери олова, получаемые при прямом сухом сжигании в муфеле, весьма значительны и колеблются в широких пределах.

Так, например, потери при сжигании воздушно-сухого хлеба с добавлением олова значительно выше (91 %), чем при сжигании воздушно-сухого рациона питания (от 35 до 51 %).

Потери при сжигании проб растительных консервов колеблются от 7 до 77,6 %, причем все олово находится в растворенном виде. В консервах животного происхождения потери олова значительно меньше — до 40,5 %, в среднем они составляют 10,5 %, но большая часть олова обнаруживается в осадке.

В пробах рыбных консервов, очевидно, благодаря содержанию в них большого количества солей кальция, препятствующих улетучиванию олова, оно зачастую открывалось полностью (даже добавленное в виде стандартного раствора $SnCl_2$), при этом все олово обнаруживалось в осадке (1300 γ; 1160 γ), а в растворе имелись его следы или оно полностью отсутствовало (0; 18; 50 γ).

Разработанный нами ранее (1957) способ переведения нерастворимых соединений олова в раствор путем электрического восстановления осадка гранулированным цинком в солянокислой среде позволил нам полностью открывать все олово, находящееся в осадке.

По Е. Б. Сандэлу (1949), олово при мокром и сухом виде сжигания превращается в β-метаоловянную кислоту, не растворимую в кислотах. Однако произведенные нами анализы различных консервов показали, что при сжигании сухим путем олово часто можно обнаружить в растворе. Специально поставленными опытами (30 определений) установлено, что нерастворимые соединения олова (SnO_2) в процессе сжигания частично превращаются в растворимые.

Наши опыты сжигания пищевых веществ без добавления ускорителей показали, что хотя в некоторых случаях мы и открывали олово полностью, главным образом в осадке, чаще оно улетучивается; кроме того, сжигание происходит медленно и неполно, с образованием спекшейся золы.

Перед нами стояла задача подыскать добавки для сухого сжигания, устраняющие потери олова и ускоряющие сжигание, а также выяснить

влияние на открываемое количество олова глазури фарфоровых тиглей, содержащей SnO_2.

Нами испытано щелочное озоление (с добавлением 0,1 н. NaOH) и озоление с добавлением ацетата магния.

Таблица 1

Сжигание пищевых продуктов с добавлением олова и ацетата магния

Наименование пищевого продукта	Навеска (в г)	Добавлено: ацетата магния	Добавлено: олова (в г)	Обнаружено: олова (в г)	[illegible]
Хлеб воздушно-сухой	5	1 мл 5%	500	366	26,3
То же	5	1 мл 10%	500	[illegible]	[illegible]
» »	5	То же	500	380	[illegible]
» »	5		200	103	[illegible]
» »	2		200	190	[illegible]
Рацион воздушно-сухой	5	4 мл 4%	500	260	[illegible]
Хлеб воздушно-сухой	5	2 мл 10%	1000	1000	[illegible]
То же	5	То же	500	483,3	[illegible]
» »	5	» »	500	500	[illegible]
» »	5	» »	500	500	[illegible]
» »	5	3 мл 10%	500	525	[illegible]
» »	5	2 мл 10%	200	196	[illegible]
» »	10	То же	200	203	[illegible]
» »	10		200	201,7	[illegible]
Мясо сырое	5	1 мл [illegible]	500	455	[illegible]
То же	5	То же	500	[illegible]	[illegible]
» »	5	2 мл [illegible]	500	541,7	[illegible]
» »	5	То же	500	505	[illegible]
» »	4		100	101,5	[illegible]
Мясо воздушно-сухое [illegible] личье	[illegible]		200	195,5	[illegible]
Молоко	[illegible]		200	196,[illegible]	[illegible]
Вермишель [illegible]	[illegible]		200	191,6	[illegible]
Капуста	[illegible]	[illegible]	500	504,5	[illegible]
Желатин	[illegible]	2 мл [illegible]	200	199,3	[illegible]
Томат	[illegible]	То же	1000	996,3	[illegible]

Попытка [illegible] щелочного озоления оказалась неудачной, [illegible] сколько результаты получались [illegible], то слишком низкими, озоление [illegible] вспучиванием веществ [illegible] образ[illegible] разрушением глазу, и фарфоровых [illegible] сжигания.

[illegible] эффективным. Для установления [illegible] магния навески пищевых [illegible] не содержащие ол[illegible] от 100 до 1000 [illegible] 1 мг/мл). В пробы доб[illegible] 11, [illegible] добавленного к не[illegible] [illegible]

Чтобы исключить возможное влияние глазури фарфоровой посуды на результаты определения олова, сжигание всех проб консервов с различными добавлениями производили одновременно в фарфоровой и кварцевой посуде. Из табл. 2 можно видеть, что при этом получаются близкие результаты.

Выводы

1. Наши исследования показывают, что мокрый способ сжигания пищевых продуктов при определении олова может быть с успехом заменен сухим озолением в присутствии лактата магния, являющегося ускорителем и связывающим олово веществом.

2. Без добавления лактата магния сухое озоление происходит чрезвычайно медленно и нередко с большими потерями олова.

3. Анализы 25 банок консервов растительного и животного происхождения показывают, что разработанный нами способ сухого сжигания в присутствии 2—3 мл 10% раствора лактата магния дает вполне приемлемые результаты. Проба сгорает быстро, с образованием рыхлой и пористой золы. Ошибка в определении олова в среднем равна ± 2,3% и не превышает ± 7,1% в отдельных случаях.

ЛИТЕРАТУРА

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METALLIC MUDDINESS OF BEER, EFFECT OF TIN IONS ON THE COLLOIDAL STABILITY OF BEER

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1. Introduction

Upon being stored from several weeks to several months, beer often loses its original clarity; it is marked by the appearance of a diffuse milky veil, a more or less thick floccule suspended in the liquid, or even a plainly distinguishable precipitate. Several factors may contribute to the formation of such muddiness; a drop in temperature causing a reversible muddiness or muddiness from cold, oxidizing substances promoting the onset of permanent muddiness, finally, some metallic ions.

Numerous works on the formation and composition of muddiness of beer emphasize the interest that this problem presents in regard to colloidal stability. We shall cite the studies of Sandegren (15), Hartong (7), St. Johnston (14), Biserte and Scriban (22), Lhoest, Lontie, and De Clerck (10), whose conclusions are sometimes not precise: the nature of muddiness still appears poorly known; we only know that

proteins, tannins, and pentoses are its principal components. More recently the works of Ljungdal and Sandegren (11), de Bangough and Harris (1) contributed some additional data on the composition of muddiness from cold.

In regard to muddiness of diverse origin, metallic muddiness presents a certain danger; the poisonous nature of salts of heavy metals, of tin in particular, was pointed out a long time ago. Luers (12), quoted by Preece (13), points out the tendency of metals to precipitate nitrogen compounds and considers tin as particularly dangerous. The works of Gray and Stone (5) on oxidation of beer and its extent direct their researches toward a comparison of the diverse forms of its muddiness. The authors note the catalytic action of copper on oxidation while pointing out that for iron and tin direct precipitation comes to the forefront. Helm (8) carries out nephelometric measurements on beer treated with Sn^{++}, Fe^{++}, Cu^{++} and admits that tin promotes permanent muddiness at the expense of reversible muddiness. Debaisieux (3) studies variations of muddiness produced by 1 mg/l Sn^{++} in terms of pH and notes on finishing his studies: "tin is dangerous for the pH of beer; it would appear that the influence of tin is not due to the greater affinity of colloids for tin, but to the fact that the isoelectric point of combination of albumin and tin is close to the pH of beer."

In fact, while all the authors are in agreement in ascribing to certain metals a role in the formation of colloidal muddiness, their opinions differ when it comes to explaining their influence. Is not the recent opinion of Heyer (9)

finely shaded? "The heavy metals like Cu, Fe, and Sn are dangerous in must and beer because they can form insoluble bonds with proteins or influence oxidation catalytically."

We have therefore tried to provide certain answers to these unresolved questions by means of a systematic study of intervening factors in the genesis of muddiness stemming from tin ions.

2. Techniques Used

Our experiments were on two light beers A and B with a density of 12.6" Balling differing in the flow of brewing tubs and the time kept. They were graciously provided by the Central Office of Lyon Breweries, to whom we offer our thanks here.

For treatment with ~~tin~~ stannous ions, we used hydrochloric solutions of ~~tin~~ stannous chloride prepared in accordance with De Clerck's technique (4).

Measurements of turbidity were made with Meunier's electrophotometer, a 72 screen, with the tub 10 millimeters thick. This method avoids the defects of Zeis's Pulfrich nephelometer, where visual comparison and the irregularity of the glass of the bottles can serve as sources of error. Our results are expressed in the scale divisions of the electeophotometer's drum reduced by the colorimetric absorption of the bottom part of the pure beer: "Meunier turbidity."

For the first beer A, a series of tests was conducted on concentrations of Sn^{++} varying from 0.05 to 2.0 mg/l. The beers containing less than 1 mg/l of Sn^{++} did not show any appreciable muddiness; the threshold of action was less elevated for beer B, being reached at only 0.2 mg/l.

We then employed concentrations of Sn^{++} of 3.5 and 10.0 mg/l for A and of 2.5 and 7.0 mg/l for B. The results are shown on the curves of figures 1 and 2, which indicate the development of turbidity in terms of time and the effect of concentrations of tin. The temperature was maintained in both cases at 17-18°C; the course of the curves is identical for both of the studied beers.

Fig. 1. Beer A. Formation of muddiness in terms of time.

(1) Turbidity (2) Days

[See page 933 of text.]

Fig. 2. Beer B. Formation of muddiness in terms of time.

(1) Turbidity (2) Days

[See page 934 of text.]

b. Influence of the Redox State

It is generally admitted that metals can promote the formation of muddiness in beers whose Redox state is modified by the addition of a reducing or an oxidizing agent. A first series of tests was conducted on beer A with variable quantities of ascorbic acid and of cysteine hydrochlorate

in order to observe the influence of the nature and the quantity of the reducing agent on the formation of tin muddiness. In the tests, the Redox state was verified by the determination of the indicator Time Test (I.T.T.)* according to the method of Gray and Stone (5). In the presence of the employed reducing agents, the beer discolors immediately the dichlorophenol-indophenol, which shows its heightened reducing power. The results are presented in Table 1.

Table 1 [see pages 935-936 of text].

1. Beer treated with 3 mg/l of Sn^{++}:
2. Ascorbic acid, mg/l
3. Meunier turbidity at the end of:
4. Day(s)
5. Cysteine, mg/l
6. Beer treated with 5 mg/l of Sn^{++}
7. Beer treated with 10 mg/l of Sn^{++}

A second series of tests was carried out on beer B treated with 2 mg/l of Sn^{++} to which had been added a portion of ascorbic acid and subsequently ammonium persulfate, a salt whose cation is not detrimental to colloidal stability: the I.T.T. is then practically infinite. The results are summarized in Table 2.

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* Indicator Time Test: Speed (in seconds) to 80 p. 100 of a standard solution of dichlorophenol-indophenol.

Table 2 [see page 936 of text].

1. Meunier turbidity
2. Time in days
3. Sample
4. Sample 1: base reference.
5. Sample 2: 1 millimol/l of ascorbic acid.
6. Sample 3: 1 millimol/l of persulfate.

c. Effect of the State of Tin Oxidation: stannic ~~Tin~~ Muddiness

We have considered several samples of beer under the same conditions as before but replaced stannous ~~tin~~ chloride with quantities of stannic chloride corresponding to 5 and 10 mg/l of tin. From the first day, there was considerable muddiness which was quite different in aspect from stannous ~~tin~~ turbidity. While the latter looked like a homogeneous veil, precipitating slowly and only in the case of strong concentrations, it formed here very rapidly a dark-brown deposit, leaving the beer on the surface quite clear. It is probable that this difference in aspect corresponds to a difference in the mechanism of formation. In any case one can conclude that the harm from Sn^{++++} is certain, although the practical interest in stannic muddiness is quite reduced.

d. Insulation and Preliminary Study of stannous ~~Tin~~ Muddiness

Several samples of beer received doses of stannous ~~tin~~ chloride ranging from 3 to 10 mg/l of Sn++. At the end of 3 to 4 weeks turbidity reached its limit vlue and the muddiness was collected by means of centrifugation. The precipitates, after being washed in distilled water, centrifuged, and dried in a vacuum, presented the appearance of a brown powder.

In a preliminary study with a complete analysis of the muddiness, we investigated the presence of tin and determined the percentage of proteins for the eventual support of the hypothesis of its formation. We calculated the percentage of proteins from the amount of nitrogen determined by the micro-kjeldahl method for variable concentrations in the tin chloride. Table 3 below shows the results.

Table 3 [see page 937 of text].

1. Sn^{++} mg/l
2. Antioxidant
3. Collected muddiness
4. Percent of nitrogen
5. Percent of proteins (N x 5.83)
6. Cysteine hydrochlorate
7. Ascorbic acid
8. Cysteine hydrochlorate
9. Ascorbic acid

We can already prove that the weight of the precipitate increases with the amount of tin, which is the reduction-oxidation state of the beer. The percentage of proteins is almost constant for the different doses of tin and allows us to predict that the process of formation of the muddiness cannot be reduced to the simple formation of a metallic proteinate.

Study of the tin in the muddiness is interesting. In fact absence of the metal favors a purely catalytic action in the formation of the muddiness; the spectrographic method used permitted tin to be detected in the examined precipitates. The same tests were repeated after dialysis of a suspension of the muddiness for 48 hours in distilled water kept at 2°C. All

the tests confirmed the presence of tin: it is thus a permanent constituent of the muddiness.

4. Discussion of the Results

The results shown above make it possible to specify certain points concerning the action of ~~tin~~ stannous salts in beer.

(1) In the first place, it is absolutely certain that the tin salts exert an intense harmful action on the beer. All the tests, with the doses varying from 2 to 10 mg/l of Sn^{++}, were positive. For smaller amounts up to 2 mg/l, the muddiness appeared more slowly or did not form at all. The samples treated with concentrations of 0.05 to 0.20 mg/l of Sn^{++} remained perfectly clear for 2 months.

(2) We established that the action of the tin salts is rapid. The curves shown on figures 1 and 2 indicate the appearance of muddiness on the first day. The muddiness increases more slowly to reach a limit value at the end of 3 to 4 weeks. Affected beer kept for several months maintained a turbidity that was practically constant. One can already eliminate the possibility of retarded action by the tin salts. Contaminated beer, when drawn off, rapidly develops ~~tin~~ stannous muddiness. If it is clear after being bottled for 24 hours, it is quite probable that there will not be any eventual muddiness from the presence of the ~~tin~~ stannous salt.

(3) Finally, it is interesting to attempt to explain the mechanism of operation of the tin in the formation of muddiness: catalytic action or direct participation of the

Sn^{++} ions. It clearly appears that it is the latter hypothesis that should be retained:

(a) The turbidity, and consequently the amount of the collected precipitate increase with the amount of added tin. But a catalytic action of the metal is denoted by the acquisition of a muddiness noticeably constant regardless of the amount of the catalyzer used.

(b) The kinetic factor in the formation of muddiness is identical for all the I.T.T. of beer from zero to infinity. It is equally independent of the nature and the quantity of the reducing agent used. But one knows that permanent muddiness is greatly diminished when one reestablishes the I.T.T. at zero by the addition of ascorbic acid, for example. Tin muddiness then certainly arises from a different mechanism and the action of the tin salts on the problem of oxidation is set aside.

(c) All the precipitates containing tin confirm its effective participation in the formation of the turbidity. One could object that colloidal ~~tin~~ stannous hydroxide is carried away by the precipitate and that dialysis is unable to separate it. This last evidence can now be discussed. It is in fact possible that tin is not found in the turbidity in a combined form, such as that of a proteinate, for example, and that it it is only attached to the surface of the muddiness. Nevertheless, in regard to this hypothesis, we can still speak of direct participation by tin. We have experienced the following: In a collodion sac, a solution of ~~tin~~ stannous chloride in a

convenient tampon is placed for dialysis against the beer, the temperature being kept around 3-4°C. In all our tests with different pH, the beer was muddied, while the neighboring beer placed under the same conditions in contact with the tampon solutions containing no tin remained clear. A part of the tin salts therefore participated in a dialyzable form in the formation of the turbidity.

(4) It is necessary to make one last comment. If the ~~tin~~ stannous salts induce muddiness in the beer, the intensity of their action varies greatly from one beer to another. Systematic tests under identical conditions have proved to us that the turbidity in a given time varies within large limits. It is probable that the condition of the constituents of the beer, the stage of protein breakdown in particular, plays an important role in the formation of tin muddiness. Sn^{++} ions have undoubtedly a special affinity for certain groupings of proteic molecules. The quantity of these functional groupings varies according to the materials first used and according to the processes of manufacture, which can explain the observed differences.

RESUME

The action of ~~tin~~ stannous chloride on beer was studied with varying concentrations of Sn^{++}. Turbidity increases with the amount of tin present and soon approaches a limit value varying with the beers used. It is remarkably constant for any given beer regardless of the Redox state of the medium.

~~Tin salts~~ Stannous salts probably exercise a direct action on the formation of the muddiness either through combination or through adsorption.

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LES TROUBLES MÉTALLIQUES DE LA BIÈRE. INFLUENCE DES IONS STANNEUX SUR LA STABILITÉ COLLOIDALE DE LA BIÈRE.

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1. — INTRODUCTION.

Après une durée de conservation variant de quelques semaines à quelques mois, la bière perd souvent sa limpidité originelle ; il apparaît un trouble se présentant sous l'aspect d'un voile laiteux et diffus, d'un floculat plus ou moins grossier en suspension dans le liquide, ou même d'un précipité nettement caractérisé. Plusieurs facteurs sont susceptibles d'intervenir dans la formation de ces troubles : l'abaissement de température provoquant le trouble réversible ou trouble au froid, les substances oxydantes favorisant l'apparition des troubles permanents, certains ions métalliques enfin.

Les nombreux travaux concernant la formation et la composition des troubles de la bière soulignent l'intérêt que présente ce problème de la stabilité colloïdale. Nous citerons les recherches de SANDEGREN [15], HARTONG [7], ST.-JOHNSTON [14], BISERTE et SCRIBAN [2], LHOEST, LONTIE et DE CLERCK [10], dont les conclusions sont parfois imprécises : la nature des troubles paraît encore mal connue, et l'on sait seulement que des protéines, des tanins et des pentoses en sont les principaux constituants. Plus récemment, les travaux de LJUNGDAHL et SANDEGREN [11], de BENGOUGH et HARRIS [1], ont apporté quelques données supplémentaires sur la composition des troubles au froid.

Parmi les troubles d'origines diverses, les troubles métalliques présentent un danger certain : la nocivité des sels de métaux lourds, de l'étain en particulier, a été signalée depuis longtemps. LUERS [12], cité par PREECK [13], remarque la tendance des métaux à précipiter les composés azotés et considère l'étain comme particulièrement dangereux. Les travaux de GRAY et STONE [5], sur l'oxydation des bières et sa mesure, orientent les recherches vers des comparaisons entre les diverses formes de troubles. Ces auteurs notent une action catalytique du cuivre sur l'oxydation, tout en remarquant que pour le fer et l'étain,

une précipitation directe vient au premier plan. HELM [8] effectue des mesures néphélométriques sur des bières traitées par Sn^{++}, Fe^{++}, Cu^{++}, et admet que l'étain favorise le trouble permanent aux dépens du trouble réversible. DEBAISIEUX [3] étudie les variations du trouble produit par 1 mg/l de Sn^{++} en fonction du pH et note en conclusion de ses recherches : « l'étain est dangereux au pH de la bière ; il semble que l'influence de l'étain n'est pas due à une plus grande affinité des colloïdes pour l'étain, mais au fait que le point isoélectrique des combinaisons albumine-étain est voisin du pH de la bière ».

En fait, si tous les auteurs s'accordent pour reconnaître à certains métaux un rôle dans la formation de troubles colloïdaux, les opinions diffèrent lorsqu'il s'agit d'expliquer cette influence. L'avis récent de HEYER [9] ne reste-t-il pas bien nuancé ? « les métaux lourds, comme Cu, Fe, Sn, sont dangereux dans le moût et la bière, parce qu'ils peuvent donner des liaisons insolubles avec les protéines ou influencer catalytiquement l'oxydation ».

Nous avons donc essayé d'apporter quelques réponses à ces questions en suspens, par une étude systématique des facteurs intervenant dans la genèse des troubles à partir des ions stanneux.

2. — TECHNIQUES UTILISÉES.

Nos essais ont porté sur deux bières blondes, « A » et « B » de densité 12,6° Balling, différant par les versements des brassins et les durées de garde. Elles nous ont été gracieusement fournies par la Centrale des Brasseries Lyonnaises que nous tenons à remercier ici.

Pour le traitement à l'ion stanneux, nous avons utilisé des solutions chlorhydriques de chlorure stanneux, préparées selon la technique de DE CLERCK [4].

Les mesures de turbidité ont été effectuées à l'électrophotomètre MEUNIER, écran 72, avec la cuve de 10 mm d'épaisseur. Cette méthode évite les défauts du néphélémètre PULFRICH de ZEISS, où la comparaison visuelle et l'irrégularité du verre des bouteilles peuvent être sources d'erreurs. Nos résultats ont été exprimés en divisions du tambour de l'électrophotomètre, diminuées de l'absorption colorimétrique de base de la bière pure : « turbidité Meunier ».

Contrôles : afin d'observer l'influence des inclusions d'air dues aux débouchages (formation possible de troubles d'oxydation), nous avons soumis des bouteilles sans étain aux mêmes manipulations. Après plusieurs semaines nous n'avons relevé aucun trouble appréciable dans ces témoins. De plus, des échantillons traités à des doses identiques d'étain présentent des troubles semblables, qu'ils aient été soumis à de nombreux prélèvements ou qu'ils soient demeurés constamment bouchés. Nous avons également procédé à l'examen microscopique d'échantillons, pour nous assurer de l'origine non bactérienne

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des troubles. Le pH mesuré avant et après addition de chlorure stanneux reste sensiblement constant ; les troubles observés n'auront donc pas leur origine dans un déséquilibre du pH de la bière.

3. — Résultats expérimentaux.

a) Sensibilité des bières et formation du trouble.

Si les avis sont unanimes sur le danger majeur présenté par l'étain, les auteurs ne sont plus d'accord sur les concentrations capables de nuire à la stabilité colloïdale. De Clerck [4] note que 0,1 mg/l peut déjà provoquer un trouble. Wixmsen, cité par Derbrieux [3], trouve que 0,25 mg/l de Sn^{++} suffit à donner un trouble, alors que Chapmann, avec des doses 250 fois plus fortes, ne remarque rien d'anormal ! Il faut bien reconnaître que la notion de seuil d'action est en elle-même subjective, les résultats n'ayant de valeur absolue que s'ils proviennent de bières identiques, traitées dans des conditions expérimentales bien définies.

Sur une première bière « A », une série d'essais a été faite avec des concentrations en Sn^{++} échelonnées de 0,05 à 2 mg/l. Après deux mois, les bières contenant moins de 1 mg/l de Sn^{++} ne présentent aucun trouble appréciable ; le seuil d'action est cependant moins élevé pour la bière « B » où il n'atteint que 0,2 mg/l.

Nous avons alors utilisé des concentrations en Sn^{++} de 3, 5 et 10 mg/l pour « A » et 2, 5 et 7 mg/l pour « B ». Les résultats sont représentés sur les courbes des Fig. 1 et 2, qui indiquent le développement de la

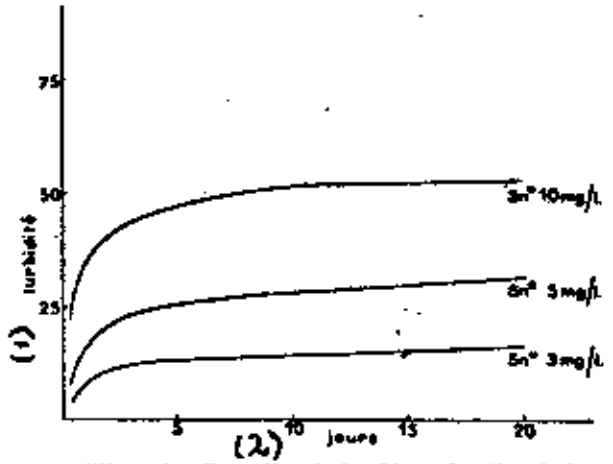

Fig. 1. — Bière « A ». Formation du trouble en fonction du temps.

turbidité en fonction du temps et l'influence de la concentration en Sn. La température est dans les deux cas maintenue à 17-18° C ; l'allure des courbes est identique pour les deux bières étudiées.

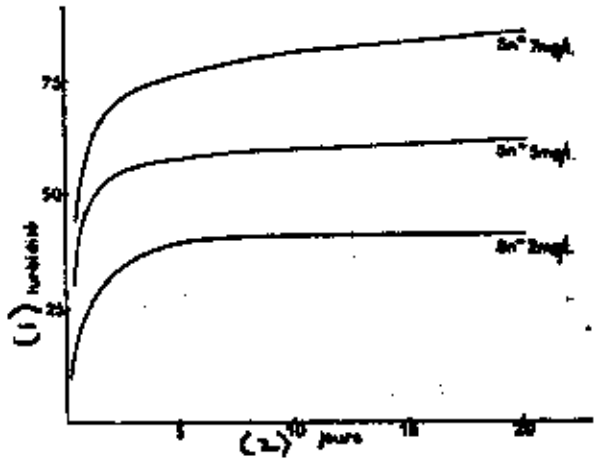

Fig. 2. — Bière « B ». Formation du trouble en fonction du temps.

b) Influence de l'état RedOx.

On admet généralement que les métaux puissent favoriser la formation du trouble permanent par action catalytique sur l'oxydation.

Pour déceler une relation éventuelle entre le trouble stanneux et le trouble d'oxydation, nous avons observé la cinétique de formation du trouble dans des bières dont l'état RedOx était modifié par addition d'un réducteur ou d'un oxydant. Une première série d'essais a été conduite sur la bière « A » avec des quantités variables d'acide ascorbique et de chlorhydrate de cystéine afin d'observer l'influence de la nature et de la quantité du réducteur sur la formation du trouble stanneux. Dans ces essais, l'état RedOx de la bière était vérifié par la détermination de l'« Indicator Time Test » (I.T.T.) (*), suivant la méthode de Gray et Stone [5]. En présence des réducteurs utilisés, la bière décolore immédiatement le dichlorophénol-indophénol, ce qui indique son pouvoir réducteur élevé. Les résultats sont rapportés dans le tableau I.

(*) Indicator Time Test : Vitesse (en secondes) de décoloration à 30 p. 100, d'une solution standard de dichlorophénolindophénol.

TABLEAU I.

(1) I. — Bière traitée avec 3 mg/l de Sn^{++} :

(2) Acide ascorbique mg/l (4)	(3) Turbidité Meunier au bout de : 1 jour	7 jours	14 jours	28 jours
0	8	14	16,5	19
20	9	16	17,5	23
40	8	12	14	20
60	8	14	16	20,5
100	8	10	14	17
(5) Cystéine mg/l				
0	8	14	16,5	19
20	7,5	12,5	17,5	23
40	8	10,5	14	20
60	8	11	16	20,5
100	8	11,5	13	16

(6) II. — Bière traitée avec 5 mg/l de Sn^{++} :

(2) Acide ascorbique mg/l				
0	18	27	31	33,5
20	20,5	31	34	38,5
40	22	31,5	35	40,5
60	18,5	30,5	35	40,5
100	21	33	35	41,5
(3) Cystéine mg/l				
0	18	27	31	33,5
20	17	27	28	30,5
40	16	24,5	26	30
60	16	25,5	27,5	31
100	16,5	28	31	34,5

TABLEAU I (suite).

(7) III. — Bière traitée avec 10 mg/l de Sn^{++} :

(2) Acide ascorbique mg/l (4)	(3) Turbidité Meunier au bout de : 1 jour	7 jours	14 jours	28 jours
0	30	46,5	50	54
20	30	50	54	60
40	30	51	56	61
60	23,5	48	51	53
100	31	40	50	57
(5) Cystéine mg/l				
0	30	46,5	50	54
20	33	44,5	50	56
40	36	52	53	55,5
60	36	52	53	56
100	40	54,5	56,5	59,5

Une seconde série d'essais a porté sur la bière « B », traitée par 2 mg/l de Sn^{++} en ajoutant d'une part de l'acide ascorbique, d'autre part, du persulfate d'ammonium, sel dont le cation est inoffensif pour la stabilité colloïdale : l'I.T.T. est alors pratiquement infini. Les résultats sont résumés dans le tableau II.

TABLEAU II.

	(1) Turbidité Meunier		
(2) Durée en jours.....	1	4	28
(3) Échantillon 1	32	50	65
Échantillon 2	33	51	63,5
Échantillon 3	42	51,5	64

(4) Échantillon 1 : témoin.
(5) Échantillon 2 : 1 millimole/l. d'acide ascorbique.
(6) Échantillon 3 : 1 millimole/l. de persulfate.

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c) Influence de l'état d'oxydation de l'étain ; trouble stannique.

Nous avons traité plusieurs échantillons de bière, dans les mêmes conditions que précédemment, mais en remplaçant le chlorure stanneux par des quantités de chlorure stannique correspondant à 5 et 10 mg/l d'étain. Dès le premier jour, il apparaît un trouble abondant, mais d'aspect tout à fait différent du trouble stanneux. Alors que ce dernier se présentait comme un voile homogène, ne précipitant que lentement et pour les fortes concentrations seulement, il se forme les très rapidement un dépôt brun-sombre laissant la bière surnageante assez claire. Il est probable qu'à cette différence d'aspect correspond une différence dans le mécanisme de formation. On peut en tout cas en conclure que la nocivité de Sn^{++} est certaine, bien que l'intérêt pratique des troubles stanniques soit assez réduit.

d) Isolement et étude préliminaire du trouble stanneux.

Plusieurs échantillons de bière reçoivent des doses de chlorure stanneux échelonnées de 3 à 10 mg/l en Sn^{++}. Au bout de 3 à 4 semaines, la turbidité atteint sa valeur limite et le trouble est recueilli par centrifugation. Les précipités lavés à l'eau distillée, centrifugés et séchés sous vide se présentent sous l'aspect d'une poudre brune.

En étude préliminaire à une analyse complète du trouble, nous avons recherché la présence d'étain et déterminé la teneur en protéines, pour appuyer éventuellement les hypothèses de sa formation. Nous avons calculé la teneur en protéine d'après la quantité d'azote déterminée par microkjeldahl, pour des concentrations variables en chlorure stanneux. Le Tableau III ci-dessous donne les résultats.

TABLEAU III.

(1) Sn^{++} : mg/l	(2) Antioxydant	(3) Trouble recueilli	(4) Azote %	(5) Protéines % (N × 5,93)
3		31,8	7,29	43,1
4		40,4	7,69	44,8
5		65	8,22	47,9
5 (6)	Chlorhydrate de cystéine	80	7,69	46,0
5 (7)	Acide ascorbique	82,5	7,63	44,5
10		122,5	7,53	44,0
10 (8)	Chlorhydrate de cystéine	80,5	7,52	43,8
10 (9)	Acide ascorbique	96	7,08	41,3

On peut déjà constater que le poids de précipité augmente avec la quantité d'étain, quel que soit l'état RedOx de la bière. La teneur en protéines est à peu près constante pour les différentes doses d'étain, et laisse prévoir que le processus de formation du trouble ne se réduit pas à la simple formation de protéinate métallique.

La recherche de l'étain dans le trouble est intéressante. En effet, l'absence du métal serait en faveur d'une action purement catalytique dans la formation du trouble ; la méthode spectrographique utilisée a permis de déceler de l'étain dans tous les précipités examinés. Les mêmes essais ont été répétés après dialyse d'une suspension du trouble pendant 48 heures, sur eau distillée maintenue à 2°. Tous les essais ont confirmé la présence de l'étain : celui-ci est donc un constituant permanent du trouble.

4. — Discussion des résultats.

Les résultats exposés précédemment permettent de préciser certains points concernant l'action des sels stanneux sur la bière :

1°) En premier lieu, il est absolument certain que les sels d'étain ont une action nocive intense sur la bière. Tous les essais, avec des doses allant de 2 à 10 mg/l en Sn^{++}, ont été positifs. Pour des teneurs inférieures à 2 mg/l, les troubles apparaissent plus lentement ou n'apparaissent pas. Des échantillons traités à des concentrations de 0,05 à 0,2 mg/l en Sn^{++} sont restés parfaitement limpides pendant 2 mois.

2°) On peut constater que l'action des sels stanneux est rapide, presque immédiate. Les courbes représentées sur les figures 1 et 2 indiquent l'apparition d'un trouble dès le premier jour. La turbidité croît plus lentement pour atteindre une valeur limite au bout de 3 à 4 semaines. Des bières troubles, conservées pendant plusieurs mois, ont gardé une turbidité à peu près constante. On peut donc déjà éliminer la possibilité d'une action-retard des sels d'étain. Une bière contaminée, lors du soutirage par exemple, donnera rapidement le trouble stanneux. Si elle est limpide 24 heures après la mise en bouteilles, il est fort probable qu'un trouble ultérieur n'aura pas pour cause la présence d'un sel stanneux.

3°) Il est intéressant enfin d'essayer d'expliquer le mécanisme d'action de l'étain dans la formation du trouble : action catalytique ou participation directe des ions Sn^{++}. Il semble bien que ce soit cette dernière hypothèse qui mérite d'être retenue :

α) La turbidité, et par suite la quantité de précipité recueilli, est une fonction croissante de la quantité d'étain ajoutée. Or une action catalytique du métal se traduirait par l'obtention d'un trouble sensiblement constant quelle que soit la quantité de catalyseur utilisée.

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b) La cinétique de formation du trouble est identique pour tous les I.T.T. de la bière de 0 à l'infini. Elle est indépendante également de la nature et de la quantité de réducteur utilisé. Or on sait que le trouble permanent est fortement diminué lorsqu'on ramène l'I.T.T. à zéro par addition d'acide ascorbique par exemple. Le trouble à l'étain procède donc certainement d'un mécanisme différent et l'action des sels d'étain sur le trouble d'oxydation est à rejeter.

c) Tous les troubles recueillis renferment de l'étain, ce qui confirme sa participation effective dans la formation du trouble. On pourrait objecter que l'hydroxyde stanneux colloïdal est entraîné par le précipité et que la dialyse est impuissante à le séparer. Cette dernière preuve peut donc prêter à discussion. Il est en effet possible que l'étain n'existe pas dans le trouble sous forme combinée, d'un protéinate par exemple, et qu'il soit seulement adsorbé sur le trouble. Cependant, dans cette hypothèse, on peut encore parler d'une participation directe de l'étain. Nous avons fait l'expérience suivante : Dans un sac de collodion, une solution de chlorure stanneux dans un tampon convenable est mise à dialyser contre de la bière, la température étant maintenue vers 3-4° C. Dans tous nos essais, à différents pH la bière s'est troublée, alors qu'une bière témoin, placée dans les mêmes conditions au contact des solutions tampons ne contenant pas d'étain est restée limpide. Une partie des sels d'étain participe donc sous forme dialysable à la formation du trouble.

4°) Une dernière remarque s'impose. Si les sels stanneux provoquent un trouble de la bière, l'intensité de leur action est très variable d'une bière à l'autre. Des essais systématiques dans des conditions identiques nous ont montré que la turbidité dans un temps donné variait dans de larges limites. Il est probable que l'état des constituants de la bière, en particulier le stade de dégradation des protéines joue un rôle important dans la formation du trouble stanneux. Les ions Sn^{++} ont sans doute une affinité particulière pour certains groupements des molécules protéiques. La quantité de ces groupements fonctionnels est variable suivant les matières premières utilisées et suivant les processus de fabrication, ce qui peut expliquer les différences observées.

Résumé.

L'action du chlorure stanneux sur la bière a été étudiée pour des concentrations variables en Sn^{++}. La turbidité, qui est une fonction croissante de la quantité d'étain, se rapproche rapidement d'une valeur limite variable suivant les bières utilisées. Elle est remarquablement constante pour une bière donnée quel que soit l'état RedOx du milieu.

Les sels stanneux ont vraisemblablement une action directe, par combinaison ou par adsorption, sur la formation du trouble.

Summary.

The action of stannous chloride on beer has been studied with varying Sn^{++} concentration. Turbidity which increases with the amount of tin, soon nears a limit value varying in accordance with the beers used. It is remarkably constant in any given beer whatever the Redox state of the medium.

Stannous salts probably have direct action on the formation of the muddiness, either by combination or by adsorption.

Zusammenfassung.

Die Aktion des Zinnchlorids auf das Bier wurde für verschiedene Konzentrationen von Sn^{++} untersucht. Die Trübung, welche eine steigende Funktion der Zinnmenge darstellt, nähert sich rasch einem für die verschiedenen untersuchten Biere wechselnden Grenzwert. Sie ist für ein bestimmtes Bier bemerkenswert konstant, welches auch immer der Redox-Zustand des Milieus sei.

Die Zinnsalze üben wahrscheinlich, durch Kombination oder Adsorption, eine direkte Aktion auf die Bildung der Trübung aus.

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HE EFFECT OF VANADIUM PENTOXIDE, FLUORIDES, AND TIN COMPOUNDS ON THE DENTAL CARIES EXPERIENCE IN RATS

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THROUGH the use of a variety of experimental conditions, it has been established that stannous fluoride behaves differently from sodium fluoride with respect to its ability to reduce experimental dental caries, both in animals[1,2] and in human beings.[3,4] However, the exact reasons for its superiority have not been established, although much previous work has indicated that the vehicle must influence its activity. In an attempt to learn the reason for this compound's apparent advantage in effectiveness over sodium fluoride, it was considered of interest to determine whether the form of the tin compound furnishing the stannous ion might contribute significantly to its superiority. Previous work has indicated that combinations of stannous fluoride with sodium fluoride and of stannous chloride with sodium fluoride were not as effective as stannous fluoride itself.[5,6] However, since the total fluoride and stannous ion concentrations were low in these experiments, this factor may restrict comparisons in animals to those between stannous fluoride and other combinations which furnish both the stannous and fluoride ions in solution. It is known, however, that the chemical state of the tin compound definitely influences the activity of stannous fluoride, since fresh solutions are more active than aged solutions.[6]

Geyer[7] has reported that vanadium pentoxide, administered to hamsters in the diet or by subcutaneous injection, inhibited dental caries to an unspecified extent. Hein and Wisotzky[8] found that hamsters receiving 10 ppm of vanadium (as V_2O_5) in the drinking water developed higher caries scores than did their littermate controls. The latter authors felt that, because of the differences in procedure, the results of the 2 studies are not necessarily contradictory. In view of these findings, it seemed interesting to investigate the effect of vanadium pentoxide on caries in the rat by the procedures used in these laboratories of administering the anticariogenic substances in the drinking water. Some consideration of possible toxic manifestations was also included, since toxicity was not discussed in the previous reports. Results with water-soluble fluoride compounds are also presented.

PROCEDURE

This experiment was divided into four separate series, since the number of animals involved was so large as to make a single study difficult to conduct

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and control adequately. In the first series, weanling Sprague-Dawley strain rats were divided into 7 groups according to sex and initial body weight. They received supplements of tin and fluoride compounds in the drinking water as shown in Table I.

In the second experiment similar weanling rats were divided into 5 groups according to sex and initial body weight as in series I. The additions to the drinking water of these animals are shown in Table III.

Series III was composed of 5 experimental groups of weanling Sprague-Dawley strain rats, each equally subdivided according to sex and initial body weight. Each group initially contained 50 animals. Group 1 received 10 μg/ml. of vanadium as vanadium pentoxide, Group 2 20 μg/ml. of vanadium, and Group 3 10 μg/ml. of vanadium. Group IV was composed of rats which received 20 μg/ml. fluoride as ammonium silicofluoride, and Group V served as a control.

In series IV, a total of 250 weanling Sprague-Dawley strain rats were divided as in series I. Groups 1 through 4 received 20 μg fluoride per milliliter as sodium hexafluorostannite, sodium fluoride, stannic fluoride or potassium fluoride, respectively. Group 5 served as a control.

All of the solutions were prepared fresh once each week, and no special precautions were taken to prevent oxidation or hydrolysis of any of the tin compounds. The extent to which differences in stability of the solutions may have influenced these results is not known. In all instances the compounds were added to the animals' drinking water, which they also received as libidum. No attempt was made to regulate the volume of water consumed by any of the animals, but visual observations indicated no major difference in water consumption by any of the different groups. All of the animals received the same stock corn cariogenic diet* which contained 0.6 μg/Gm. of fluorine. The animals were housed in pairs in raised screen cages, in an air-conditioned room. The duration of the experiments was 140 days, after which time the animals were sacrificed by ether and the heads were examined for dental caries as previously described.[6] Four representative male and female rats from each group were analyzed for total fluorine in the carcass according to procedures previously described.[7] The animals used for fluorine analysis from each group were selected in such a manner that their final weights were all comparable.

DATA AND DISCUSSION

The anticariogenic effects of the various tin compounds used in series I are seen in Table I. Results of statistical analysis (Table II) show that neither stannous gluconate nor stannous chloride caused a significant reduction in caries incidence, as compared with the controls. These observations are not inconsistent with those previously reported,[10] which showed that the stannous ion alone, without fluoride, had only a slight anticariogenic effect. The marked effect of fluorine is demonstrated by a significant difference in the dental caries

*Yellow corn grits, 52.7%; ground yellow corn, 11.3%; powdered whole milk, [illegible]; alfalfa, 1.8%; iodized sodium chloride, 1.0%; and irradiated yeast, 0.2%.

TABLE I

A 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* (%)
1	Stannous gluconate†		90	25	7.2	8
2	Stannous gluconate (plus fluorine‡)	30	90	26	4.5	42
3	Stannous chloride		90	29	6.9	42
4	Stannous chloride (plus fluorine‡)	30	90	33	4.9	37
5	Stannous fluoride§	30	90	30	3.7	53
6	Stannous chloride		180	19	6.4	18
7	None	—	—	33	7.8	—

*Based upon number of carious lesions.

†Furnished by the Metal and Thermit Research Laboratory, Rahway, N. J.

‡As sodium fluoride.

§Prepared at Indiana University by Mr. Joseph Davis to whom we are indebted. See J. Am. Chem. Soc. 71: 1561, 1952, for method of preparation.

TABLE II

STATISTICAL ANALYSIS OF SERIES I DATA

Treatment Groups

1. Stannous gluconate, 90 ppm Sn++
2. Stannous gluconate, 90 ppm Sn++; NaF, 30 ppm F
3. Stannous chloride, 90 ppm Sn++
4. Stannous chloride, 90 ppm Sn++; NaF, 30 ppm F
5. Stannous fluoride, 30 ppm F and 90 ppm Sn++
6. Stannous chloride, 180 ppm Sn++
7. Control

ALL POSSIBLE PAIRS	TREATMENT DIFFERENCE	SEPARATION OF TREATMENT BY Q TEST†	
		0 (.05)‡	0 (.01)
1-2	2.78	2.42	2.80
1-3	0.31	2.42	—
1-4	2.87	2.09	—
1-5	3.51	2.42	2.81
1-6	0.71	2.42	—
1-7	0.55	2.42	—
2-3	2.11	2.42	2.10
2-4	0.51	2.42	—
2-5	0.76	2.42	—
2-6	2.07	2.10	—
2-7	2.33	2.42	2.84
3-4	1.93	2.01	—
3-5	3.20	2.42	2.81
3-6	0.37	2.42	—
3-7	0.89	2.42	—
4-5	1.27	2.42	—
4-6	1.56	2.42	—
4-7	2.82	2.42	2.84
5-6	2.83	2.42	2.84
5-7	1.09	2.42	2.84
6-7	1.26	2.42	—

*The statistical analysis was performed by Mr. Robert Lehnhoff to whom we are indebted.

†Scheffe, H.: A Method for Judging All Contrasts in the Analysis of Variance, Biometrika, 40: 87-104, 1953.

‡If a treatment difference is as large as or larger than the decile, a significant difference can be said to exist at the probability level associated with the decile.

experience when 30 ppm F as sodium fluoride is added to the stannous gluconate or stannous chloride solution. While the caries figures were not significantly different for the animals receiving stannous fluoride and for those receiving stannous gluconate or stannous chloride plus sodium fluoride, the trend shown here do not contradict the previously reported observation that mixtures of $SnCl_2$ and NaF are less effective than is SnF_2 in reducing dental caries in rat.[7]

The dental caries experience in the series II animals is seen in Table III.

TABLE III
A COMPARISON BETWEEN THE DENTAL CARIES EXPERIENCE OF RATS RECEIVING DIFFERENT FLUORIDE COMPOUNDS IN THEIR DRINKING WATER (SERIES II)

GROUP NO.	SUPPLEMENT	FLUORIDE CONC. (µg/ML)	TIN CONC. (µg/ML)	NO. OF RATS	MEAN NO. OF LESIONS	CARIES PREVENTION* (%)
1	Potassium stannous fluoride† (potassium fluorostannite)	30	60	26	5.6	[illegible]
2	Stannous fluoride‡	30	90	29	5.0	[illegible]
3	Stannous fluoride plus sodium fluoride	30	45	31	7.2	[illegible]
4	Stannous fluoride plus potassium fluoride	30	45	28	6.8	[illegible]
5	None			30	8.4	

*Based upon number of carious lesions.
†Furnished by Dr. William E. Bruce, 900 North Broad Street, Philadelphia, Pa., to whom we are indebted.
‡Prepared at Indiana University by Mr. Gino Ciccarelli to whom we are indebted.

The effect of potassium stannous fluoride (potassium fluorostannite) is quite similar to that of stannous fluoride with both producing a significant reduction (Table IV) in the dental caries experience. However, mixtures of stannous fluoride with potassium or sodium fluoride at the same fluorine concentration as furnished by stannous fluoride or potassium stannous fluoride are less effective. These data also indicate that there may be inherent anticariogenic properties possessed by stannous fluoride which are not duplicated by mixtures furnishing similar ions in solution. It cannot be stated at this time whether the difference results from the lowered tin concentration or from an inherent difference in anticariogenic properties between the 2 kinds of solutions.

The amount of fluorine stored in the rat from the different fluorides as determined by total carcass analysis in the series I animals is seen in Table V and the results for series II are in Table VI. While fluoride intake figures were not obtained in this study, there was no gross indication that the groups differed significantly in fluid consumption. The females stored less fluorine than the males, except in the control groups. That the females stored more fluorine in the skeleton than the males when the intake was low (less than 3 µg/Gm.) corroborates previous work.[11] Similarly, there is no apparent relationship between the amount of fluoride stored and the effect of the compound on the dental caries rate. The data do have a tendency to indicate, however, that the presence of the stannous ion as furnished by either stannous fluoride or stannous chloride interferes with the storage of fluorine in the skeleton when compared to similar

concentrations of fluorine in the presence of stannous gluconate. This is in keeping with other experimental studies which indicate that stannous gluconate does not hydrolyze as rapidly as stannous chloride.

TABLE IV
STATISTICAL ANALYSIS OF SERIES II DATA*

Treatment Groups
1. $KSnF_3$; 30 ppm F
2. SnF_2; 30 ppm F
3. SnF_2 + NaF; 30 ppm F
4. SnF_2 + KF; 30 ppm F
5. Control

Comparison of Treatment Groups	Q Statistic (Q Criterion)	Significance of Treatment by Q Test	
		p (.05)	p (.01)
1-2	0.68	1.70	–
1-3	1.70	1.68	–
1-4	1.25	1.73	–
1-5	2.93	1.72	2.02
2-3	1.78	1.68	–
2-4	1.33	1.73	–
2-5	3.61	1.72	2.02
3-4	0.45	1.72	–
3-5	1.93	1.70	–
4-5	1.68	1.71	–

* See Table V for footnotes.

TABLE V
TOTAL FLUORIDE IN RAT CARCASSES (SERIES I ANIMALS)

Group No.	Supplement	Total F (Mg.) Females	Total F (Mg.) Males
1	Stannous gluconate (30 μg Sn/ml.)	0.13 ± 0.11*	0.31 ± 0.01*
2	Stannous gluconate plus sodium fluoride	22.17 ± 2.03	19.73 ± 2.05
3	Stannous chloride (30 μg Sn/ml.)	0.51 ± 0.12	0.13 ± 0.07
4	Stannous chloride plus sodium fluoride	16.85 ± 1.50	12.98 ± 3.02
5	Stannous fluoride	18.16 ± 2.00	16.51 ± 1.71
6	Stannous chloride (150 μg Sn/ml.)	0.42 ± 0.08	0.31 ± 0.01
7	None	0.26 ± 0.02	0.51 ± 0.03

* Standard deviation.

TABLE VI
TOTAL FLUORIDE IN RAT CARCASSES (SERIES II ANIMALS)

Group No.	Supplement	Total F (Mg.) Males	Total F (Mg.) Females
1	Potassium stannous fluoride	21.9 ± 1.3*	16.8 ± 1.6
2	Stannous fluoride	18.7 ± 3.1	18.0 ± 0.9
3	Sodium fluoride and stannous fluoride	23.5 ± 3.0	17.7 ± 2.3
4	Potassium fluoride and stannous fluoride	20.0 ± 2.5	17.5 ± 2.0
5	None	0.3 ± 0.03	0.32 ± 0.02

* Standard deviation.

The data obtained from the series III animals are found in Table VII. These data indicate that none of the groups of animals which received the solutions of vanadium pentoxide had any reduction in their dental caries incidence. There was a numerical, although not significant, increase in caries in the 2 groups surviving at the end of the experimental period. The group which received the ammonium fluorosilicate had an apparent decrease in dental caries but it also was not significant.

The animals which received the vanadium pentoxide were highly affected toxicologically by the solution. Those which received the highest concentration (40 μg/ml. vanadium) all died within 65 days after first receiving the solution. Some of the animals receiving the less concentrated solution survived the experimental period but in each instance the toxicity of the solution was manifested both by an increase in mortality and by smaller body weight of the survivors.

TABLE VII
THE EFFECT OF VANADIUM PENTOXIDE AND AMMONIUM SILICOFLUORIDE ON THE DENTAL CARIES EXPERIENCE IN RATS (SERIES III)

COMPOUND	VANADIUM CONC. (μg/ML.)	FLUORIDE CONC. (μg/ML.)	SEX	NO. OF RATS AT TERMINATION OF EXPERIMENT	WEIGHT GAIN (GM.)	MEAN NO. OF LESIONS	
Vanadium pentoxide	40	-	all animals died				
Vanadium pentoxide	20	-	Male	15	188	9.5	9.2*
			Female	12	123	8.9	
Vanadium pentoxide	40	-	Male	11	212	9.0	8.6
			Female	16	111	8.1	
Ammonium silicofluoride		20	Male	20	236	7.9	7.7
			Female	20	118	7.5	
Water			Male	20	25[illegible]	[illegible].7	8.3
			Female	19	115	7.9	

*Sexes combined.

It is not easy to compare these findings with those of Geyer[4] since he did not mention the effect of the vanadium on the growth of the animals. However, no dental caries reductions were noted in our studies, these findings being similar to those reported by Hein and Wisotzky[5] in the hamster.

In all instances the groups of series IV animals receiving the 4 different fluoride solutions had a directional tendency for less dental caries as compared to the control group (Table VIII). However, only stannic fluoride and sodium fluoride led to significant reductions, but they were significant only at the 0.08 level of confidence. This latter point is of interest since stannous fluoride has been shown to have a highly significant effect on dental caries reduction in both the rat[13-14] and the hamster.[3] Apparently, the tin moiety must be present in the stannous state in order to obtain maximum effectiveness from tin fluoride solutions. These data corroborate previous observations in rats[6] which indicated that freshly prepared solutions decrease the dental caries rate to a greater degree than aged solutions. These findings would also

suggest that solutions of stannous fluoride which are to be used for topical applications should be made and used fresh in order to obtain the maximum effectiveness. However, since the chemistry of tin and fluorine compounds is so complicated, additional studies are needed in this area concerning the effect of oxidation and hydrolysis on the effectiveness of stannous fluoride in inhibiting both human and experimental animal dental caries.

TABLE VIII
A COMPARISON BETWEEN THE DIFFERENT FLUORIDES AND A CONTROL GROUP RECEIVING DISTILLED WATER ON THE INCIDENCE OF DENTAL CARIES (SERIES IV)

	FLUORIDE CONC. (μg/ml.)	SEX	NUMBER OF SURVIVING ANIMALS	WEIGHT GAIN (GM.)	DENTAL CARIES EXPERIENCE: MEAN NO. OF LESIONS		EXTENT	MOLARS AFFECTED
Sodium hexafluorostannite	20	Male	21	265	9.0	> 8.5*	1.77	5.51
		Female	20	161	8.0		1.59	5.07
Sodium fluoride	20	Male	18	267	8.1	> 7.7	1.52	5.53
		Female	20	148	7.2		1.37	5.11
Stannic fluoride	20	Male	21	297	8.1	> 7.6	1.73	5.31
		Female	20	152	6.7		1.26	
Potassium fluoride	20	Male	22	271	8.3	> 8.3	1.62	5.22
		Female	21	145	8.3		1.70	5.28
Control		Male	17	271	9.9	> 9.6	2.43	5.50
		Female	19	136	9.3		2.07	5.56

*Sexes combined.

SUMMARY

Stannous fluoride (alone and with sodium or potassium fluoride), stannous chloride (alone and with sodium fluoride), stannous gluconate (alone and with sodium fluoride), and potassium fluorostannite were administered to weanling rats in the drinking water for 140 days. Dental caries rates were significantly reduced by stannous fluoride, by potassium fluorostannite, and by stannous chloride and stannous gluconate to which sodium fluoride was added. The stannous salts alone (without fluoride) were essentially without effect. Stannous fluoride supplying fluoride at a level of 30 μg/ml. was more effective than an equimolecular mixture of stannous fluoride with either sodium or potassium fluoride, which furnished fluoride at the same level; in the latter cases the tin level was, of course, reduced by 50 per cent.

The effectiveness of vanadium pentoxide, ammonium silicofluoride, sodium hexafluorostannite, sodium fluoride, stannic fluoride, and potassium fluoride were studied for their anticariogenic effectiveness in rats. None of the solutions significantly reduced the incidence of dental caries except stannic fluoride and sodium fluoride, and these were significant only at a low level of confidence. The vanadium pentoxide solutions were highly toxic as evidence by the failure of the rats to gain weight normally as well as by the high mortality during the study.

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MOTOR END PLATE REACTIVITY TO DIVALENT METAL IONS HISTOCHEMICAL STUDIES[1]

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Motor end plates in the tibialis anterior muscle of the rat were demonstrated by metal sulfide deposits following injection of aqueous solutions of lead, stannous, cadmium, zinc or cupric ions into the muscle *in vivo* or *in vitro*. The appearance of the end plates was similar to the structure demonstrated by cholinesterase staining, with visualization of the subneural apparatus. Neither metal binding nor cholinesterase activity was affected 4 weeks after dissection of the sciatic nerve, indicating that the metal binding site is postsynaptic. Freezing or formalin fixation of muscle prevented binding of all metal ions to the end plate without greatly affecting cholinesterase activity, indicating that these two activities of the end plate are distinct. Prior administration of acetylcholine, *d*-tubocurarine, neostigmine or diisopropyl fluorophosphate inhibited binding to the end plate of cadmium and zinc ions but did not alter binding of lead and stannous ions. By formation of a lake with alizarin red S previously injected *in vivo* intramuscularly, the release of calcium ions at the motor end plate following stimulation of the muscle through the nerve or administration of neostigmine was demonstrated. These results suggest a close relationship of the site of binding of divalent metal ions in the motor end plate to the site of calcium release, and a close but not identical relationship to the site of cholinesterase activity and the acetylcholine receptor.

Sávay and Csillik (18, 19) and Csillik (5) demonstrated that lead ions bind to the subneural apparatus of motor end plates following intramuscular injection of lead nitrate solution *in vivo* or immersion of freshly excised muscle into lead nitrate solution. The appearance of the end plate stained with lead sulfide resembles the appearance produced by staining for cholinesterase (11), and both the lead binding and cholinesterase activities persisted for many weeks after denervation, indicating that they are located in the postsynaptic (muscle) rather than presynaptic (motor nerve terminal) membrane. However, these activities of the motor end plate appear to be distinct, since formalin fixation or freezing of muscle destroyed the lead binding activity, but did not affect cholinesterase activity (19).

In the current study, the lead-binding activity of the motor end plate of the striated muscle of the rat has been studied, and compared with its binding activity with stannous, cadmium, zinc, cupric, cobaltous, ferrous and mercuric ions.

MATERIALS AND METHODS

Muscle: The tibialis anterior muscles from male rats of the Wistar strain, weighing 100-450 [illegible], were used. A total of 240 muscles of 120 rats [illegible] studied.

Demonstration of binding of divalent metal ions to motor end plates *in vivo*: This procedure is a modification of the method described by Sávay and Csillik (19). Solutions of divalent metal ions and their concentrations and pH used in this study were: 5% (w/v) lead nitrate ($Pb(NO_3)_2$) in distilled water (pH 4.25) or in 0.035 *M* Veronal acetate buffer (final pH 5.0); saturated aqueous solution of lead chloride ($PbCl_2$), approximately 1% (w/v) (pH 4.1); 5% (w/v) aqueous solution of lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$) (pH 6.4); [illegible] (w/v) stannous chloride ($SnCl_2 \cdot 2H_2O$) in 0.05 [illegible] hydrochloric acid (pH 1.3); 5% (w/v) cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2}H_2O$) in 0.035 *M* Veronal acetate buffer (final pH 6.7); 5% (w/v) zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$) in 0.035 *M* Veronal acetate buffer (final pH 6.5); 5% (w/v) cupric sulfate ($CuSO_4 \cdot 5H_2O$) in 0.035 *M* Veronal acetate buffer (final pH 2.8); 5% (w/v) aqueous solution of co[illegible]

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ion nitrate ($Co(NO_3)_2 \cdot 6H_2O$) (pH 5.2); 5% (w/v) aqueous solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) (pH 1.0); 5% (w/v) aqueous solution of mercuric nitrate ($Hg(NO_3)_2 \cdot H_2O$) (pH 1.9); and 5% (w/v) aqueous solution of mercuric chloride ($HgCl_2$) (pH 2.5). The pH of these solutions was lower than the pH of tissues as the solubility of metal compounds was small at neutral pH.

Rats were anesthetized by intraperitoneal injection of pentobarbital solution, and the tibialis anterior muscle was exposed. The metal ion solution, 0.3 ml/100 g body weight, was injected into the muscle through a 27-gauge needle inserted in the distal third of the muscle and moved proximally during the injection toward the middle third of the muscle, where the end plates are located. The muscle was exercised 20 min after injection, and frozen in isopentane-Dry Ice for 30 sec. Frozen sections of muscle of 20 μ thickness were made by a microtome in a cryostat at −17°C. The sections were placed on a cover slip, thawed in formalin vapor at room temperature for about 10 sec and then washed in distilled water for 10 sec. Lead, cadmium, zinc, cupric, cobaltous and mercuric ions in the section were visualized as sulfide deposits by immersing the sections in 2% (w/v) sodium sulfide ($Na_2S \cdot 9H_2O$) solution in 70% (v/v) ethanol. The sections were then placed in 3% (w/v) aqueous solution of aluminum potassium sulfate for 30 sec, rinsed in distilled water for 2 min and mounted on a glass slide with a mixture of equal parts of glycerine and distilled water. For visualization of stannous ions, the sections were immersed in 2% (w/v) sodium sulfide in 0.2 N acetic acid for 2 sec, and treatment with aluminum potassium sulfate solution was omitted. In order to demonstrate ferrous ions in the sections, 0.3 ml fresh mixture of equal parts of 2% (w/v) aqueous solution of potassium ferrocyanide and 2% (w/v) aqueous solution of hydrochloric acid was injected intramuscularly at the same site 20 min after injection of ferrous sulfate solution. The muscle was frozen sectioned, rinsed and mounted on a glass slide. For the detection of mercuric ions, the sections of muscle were treated either with sodium sulfide in ethanol and aluminum potassium sulfate, or with 1% (w/v) diphenylcarbazone in 90% (v/v) ethanol.

Demonstration of calcium by staining with alizarin red S: This procedure was modified from the method of Gillik and Silvay (7). One percent (w/v) aqueous solution of alizarin red S was injected *in vivo* into rat tibialis anterior muscle, in the same manner as the solutions of metal ions. The muscle was immediately excised, and frozen (isopentane-Dry Ice for 30 sec. Frozen sections (20 μ thickness were thawed on a cover slip in formalin vapor at room temperature for about 10 sec, washed in absolute ethanol for 5 min, cleared in xylene and mounted in balsam.

Demonstration of the motor end plates by staining for cholinesterase activity: This procedure was carried out by a modification of the acetylthiocholine method of Koelle and Friedenwald (11). Fresh frozen sections of muscle were incubated for 60 min at room temperature in the substrate solution containing 8 ml supernatant from a mixture of 7.8 ml aqueous solution of 0.15 g acetylthiocholine iodide and 3.6 ml aqueous solution of 0.06 g cupric sulfate ($CuSO_4 \cdot 5H_2O$), and 92.2 ml 0.05 M Veronal-acetate buffer (pH 4.9) with 0.075 g glycine and 0.05 g cupric sulfate. The sections were rinsed in distilled water for 30 sec and then placed in 2% (v/v) ammonium sulfide solution for 20 sec for visualization of cholinesterase activity. The sections were washed in distilled water, dehydrated with ethanol, cleared with xylene and mounted on a glass slide in balsam.

In muscle which had been injected with metal ion solution, cholinesterase activity was visualized, after incubation of sections in the substrate solution, by immersing in an aqueous solution containing 5 g potassium ferricyanide ($K_3Fe(CN)_6$) and 15 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) in 100 ml (100). This is because ammonium sulfide treatment resulted in visible sulfide deposits where either metal ions or cholinesterase activity is located.

Demonstration of nerve axons and cholinesterase activity: The demonstration in the same section of nerve axons by the silver method and cholinesterase activity by the acetylthiocholine method was carried out as reported in a previous communication (14).

In order to examine the distribution of motor end plates in the tibialis anterior muscle of rats, serial frozen sections of muscle in frontal, sagittal and horizontal planes were stained for cholinesterase activity, or for both cholinesterase activity and nerve axons.

Factors affecting binding of metal ions and cholinesterase activity of end plates: The effect of prior freezing, formalin fixation or denervation of muscle and of prior treatment with compounds which affect neuromuscular transmission was studied. Excised tibialis anterior muscle was frozen in isopentane-Dry Ice for 30 sec and thawed for about 30 min at room temperature. Formalin-fixed muscle was prepared by immersing the muscle in 10% (v/v) formalin in 0.9% (w/v) sodium chloride solution for 6 hr at 4°C. Denervation was carried out 2 or 4 weeks prior to experiments by removing about 1 cm of the sciatic nerve. Treatment with compounds which affect neuromuscular transmission was carried out by injection into the tibialis anterior muscle of the following

solutions; 0.5 ml 100 *mM* acetylcholine bromide in 0.85% sodium chloride 5 min prior to the injection of metal ions; 0.5 ml 10 *mM* *d*-tubocurarine chloride in 0.85% sodium chloride or 0.1 ml injected into the femoral artery 5 min prior to the injection of metal ions; 0.5 ml 1 *mM* neostigmine methylsulfate in 0.85% sodium chloride 15 min prior to the metal ion injection or 0.2 ml injected intraperitoneally 10 min prior to the metal ion injection; and 0.5 ml 1 *mM* diisopropyl fluorophosphate in 0.85% sodium chloride 30 min prior to the injection of metal ions.

RESULTS

Demonstration of end plates in the tibialis anterior muscle of the rat by staining for cholinesterase activity: All motor end plates of the rat tibialis anterior muscle were found in the middle third of the muscle. The end plates of muscle fibers on the surface of the muscle were located more distally than end plates of fibers deeper in the muscle, resulting in a dome-shaped distribution of the end plates, with the apex of the dome directed proximally (Fig. 1). The end plates were arranged in a parabolic line in longitudinal (frontal or sagittal) sections of the muscle (Fig. 2), and in an open or closed circle in cross-sections (horizontal) of the middle third of the muscle (Fig. 3). This distribution of the end plates appeared to be due to differences in the length of the muscle fibers, which determined the location of the midpoint of the muscle fiber where the end plate is localized. The end plates of the more superficial muscle fibers were located more distally, as these fibers were longer, terminating more distally at the tendon of insertion than the deeper shorter muscle fibers.

At higher magnification, the motor end plate showed a subneural apparatus with net-like structure, and palisade-like finer lines perpendicular to the net-like structure, probably corresponding to the secondary synaptic clefts (Fig. 4).

The nerves and blood vessels entered the muscle from the proximal portion of the posterior surface and the nerve fibers formed a dome as they approached the end plate zone.

Demonstration of end plates by binding of divalent metal ions *in vivo*: In sections of the tibialis anterior muscle which received an injection of lead nitrate *in vivo* and were then processed as described, the end plates were visualized by brown-black deposits of lead sulfide. Their distribution and structure were almost identical with those of motor end plates demonstrated by staining for cholinesterase, including the presence of palisade-like secondary synaptic clefts (Fig. 5). When an aqueous solution of 5% lead nitrate was utilized instead of a buffer solution, the deposit of lead sulfide appeared to be less dense. The end plates could also be visualized with lead acetate (Fig. 6) or lead chloride (Fig. 7).

The subneural apparatus of the motor end plate was also demonstrated as metal sulfide deposits following injection *in vivo* into rat tibialis anterior muscle of solutions of other divalent metal cations. The subneural apparatus appeared brown-black following injection of stannous chloride solution (Fig. 8), gold yellow following injection of cadmium chloride solution (Fig. 9), silver-gray following injection of zinc acetate solution (Fig. 10) and brown-black following injection of cupric sulfate solution (Fig. 11). The structures of the subneural apparatus demonstrated by binding of these ions was the same as that visualized with lead. The binding of these metal ions with the motor end plate could also be demonstrated following injection of 1% (w/v) solution of metal compounds, instead of the 5% solutions described under "Materials and Methods."

The pH of the solutions of lead, stannous and cupric ions injected was relatively low in all instances. However, there was no evidence of binding of cadmium or zinc ions when solutions of these ions in 0.05 *N* hydrochloric acid (pH 1.5) were injected. Binding of aqueous solutions of cobalt,

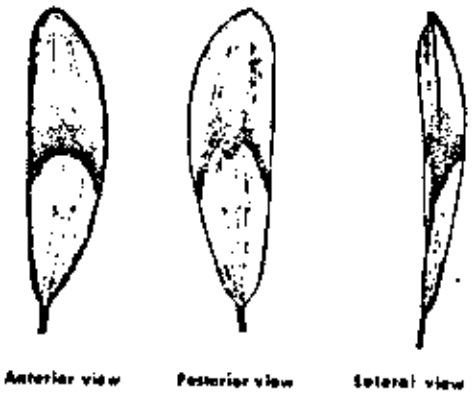


FIG. 1. Diagrammatic representation of distribution of motor end plates in rat tibialis anterior muscle. The heavy dots indicate the position of end plates on or near the surface of the muscle, and the lighter dots indicate end plates deeper within the muscle. The end plates are localized in the middle third of the muscle. End plates on or near the surface are situated more distally than those within the muscle, resulting in a dome-shaped distribution of the end plates. The top of the muscle is the origin (proximal), and the bottom is the insertion (distal).

FIGS. 2 and 3. The motor end plates of rat tibialis anterior muscle. Frontal (longitudinal) section (origin of muscle (proximal end) toward the left (Fig. 2)) and horizontal (cross) section, with anterior surface of muscle at the top (Fig. 3). Cholinesterase staining. ×4.

FIG. 4. The motor end plate of rat tibialis anterior muscle. Cholinesterase staining. ×775.

FIGS. 5–11. The motor end plates of rat tibialis anterior muscle. Visualized as metal sulfide deposits following intramuscular injection *in situ* of solutions of metal compounds. Injected compounds and color of sulfide deposits are: lead nitrate, brown-black (Fig. 5); lead acetate, brown-black (Fig. 6); lead chloride, brown-black (Fig. 7); stannous chloride, brown-black (Fig. 8); cadmium chloride, gold-yellow (Fig. 9); zinc acetate, silver-gray (Fig. 10); and cupric sulfate, brown-black (Fig. 11). ×775.

ferrous or mercuric ion could not be demonstrated by the procedure described in this method. It is possible that alterations in the procedure, particularly neutralization of the strongly acid solutions of these ions, may enable binding to be demonstrated.

Demonstration of end plates by binding of divalent metal ions with excised rat tibialis anterior muscle: Binding of metal ions with the subneural apparatus was also demonstrated when solutions of lead, stannous, cadmium, zinc or cupric ions were injected into freshly excised muscle. When muscle was stored at 1°C for 24 hr prior to injection of the metal ion, binding activity was much lower. When muscle was frozen and thawed prior to injection, no binding of any of the metal ions with the end plate could be demonstrated, although cholinesterase staining was well preserved. Similarly, prior fixation of muscle with formol-saline completely inhibited the binding of each of the metal ions, while cholinesterase staining was only slightly decreased.

Properties of end plates after binding of divalent metal ions: Bound lead could not be removed by rinsing of muscle sections in distilled water for 24 hr. Digestion of muscle sections with trypsin did not affect the lead ions bound to the end plate, although myofibrils and other muscle structures were destroyed (Fig. 12). Trypsin digestion was performed by incubating muscle sections at 37°C for 30 min in 0.01% (w/v) trypsin (10,000 benzoyl-arginine ethyl ester units/mg) in phosphate-buffered saline (pH 7.2). Bound lead was removed by treatment of muscle sections with 0.5% (w/v) aqueous solution of disodium ethylenediaminetetraacetate for 15 min. Bound cadmium ions, on the other hand, were removed simply by rinsing the muscle sections in distilled water for 2 hr. In these experiments, demonstration of bound metal ions by immersing the samples in sulfide solution was performed after treatment with water, trypsin or ethylenediaminetetraacetate.

Cholinesterase activity was demonstrated in the end plate following binding of lead ions (Fig. 13) or following removal of lead by treatment with disodium ethylenediaminetetraacetate solution, although the activity was considerably lower in each instance than in the end plate of untreated muscle.

Effect of denervation and of compounds which affect neuromuscular transmission on the binding of divalent metal ions to end plates: Denervation of the sciatic nerve 2 to 4 weeks prior to the experiment did not affect the affinity of the subneural apparatus for divalent metal ions (Figs. 14 and 15), although the shape of the end plate seemed to have become irregular. At these stages of denervation, the nerve axon could not be demonstrated, while cholinesterase activity of the end plate was intact (Figs. 16 and 17).

The prior local intramuscular injection of acetylcholine or *d*-tubocurarine (Fig. 18) or the intra-arterial injection of *d*-tubocurarine had no effect on the binding of lead or stannous ions with the end plate. Likewise, the prior local injection of diisopropyl fluorophosphate (Fig. 19) or neostigmine (Fig. 20), or the intraperitoneal injection of neostigmine, in doses which produced muscular fasciculations and complete inhibition of cholinesterase activity of the end plate, did not alter the binding of these ions. On the other hand, the prior administration of acetylcholine, *d*-tubocurarine, neostigmine or diisopropyl fluorophosphate prevented the binding of cadmium or zinc ions to the end plate.

Demonstration of calcium release at the neuromuscular junction *in vivo*: Immediately after tetanic stimulations of the tibialis anterior

FIG. 12. The motor end plate of rat tibialis anterior muscle digested with trypsin following intramuscular injection *in vivo* of lead nitrate solution. Demonstrated as lead sulfide deposits. ×775.

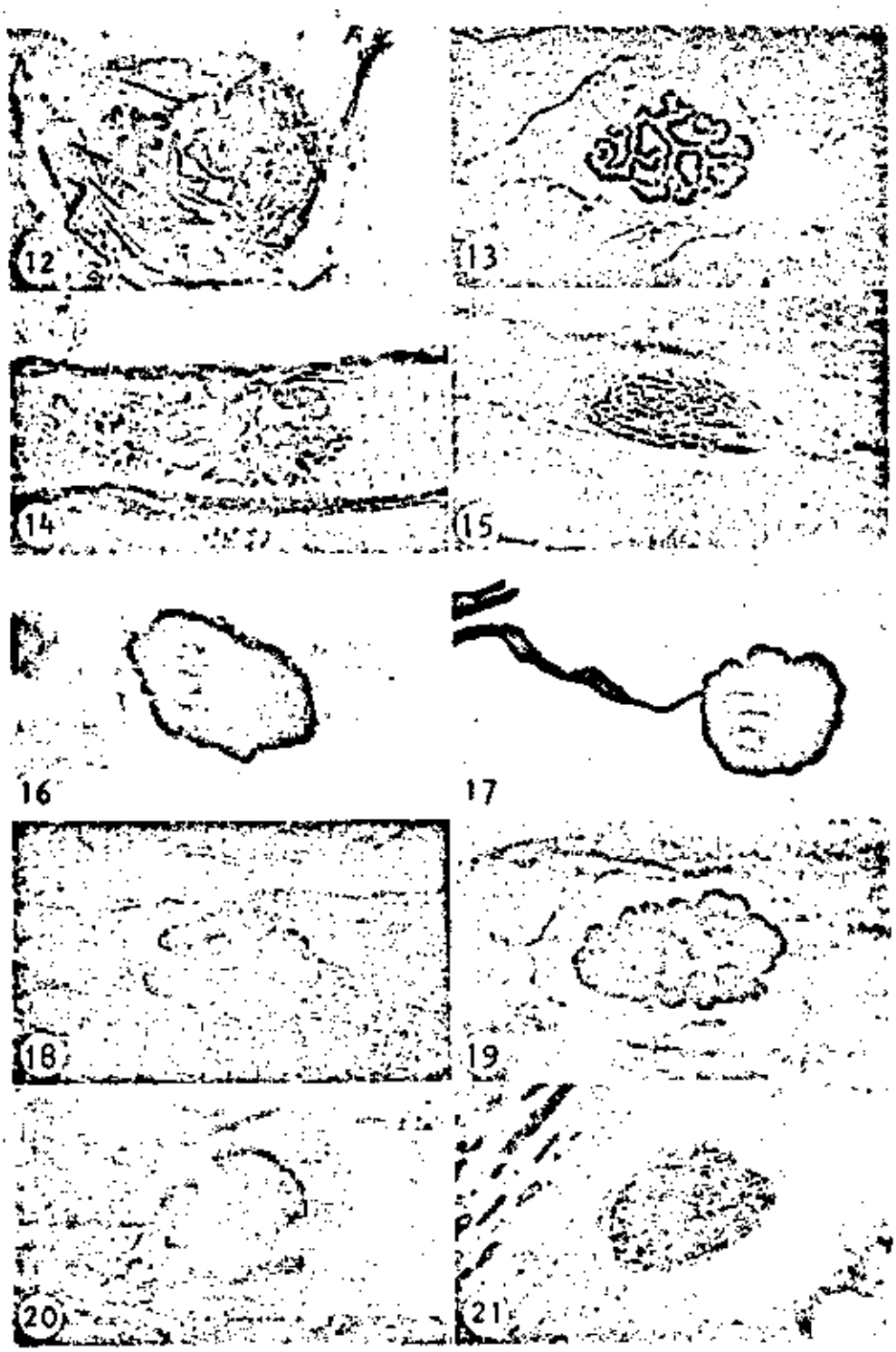
FIG. 13. Cholinesterase activity of the motor end plate of rat tibialis anterior muscle which had been bound with lead ions by intramuscular injection *in vivo* of lead nitrate solution. Cholinesterase staining. ×775.

FIGS. 14 and 15. The motor end plate of rat tibialis anterior muscle 2 weeks after denervation. The motor end plate was demonstrated as metal sulfide deposits following intramuscular injection *in vivo* of stannous chloride (Fig. 14) and cadmium chloride (Fig. 15). ×775.

FIGS. 16 and 17. The motor end plate of rat tibialis anterior muscle demonstrated by staining for nerve axon and cholinesterase activity. Presence of cholinesterase activity and absence of nerve axon in muscle 2 weeks after denervation (Fig. 16), in contrast to the demonstration of both cholinesterase activity and the nerve axon in control muscle (Fig. 17). ×775.

FIGS. 18-20. Failure of compounds which affect neuromuscular transmission to alter the binding of lead and stannous ions with the end plate, visualized as metal sulfide deposits. Stannous chloride solution injected after the intramuscular administration of *d*-tubocurarine (Fig. 18) or diisopropylfluorophosphate (Fig. 19), and lead nitrate solution injected after intramuscular administration of neostigmine (Fig. 20).

FIG. 21. Calcium ions at the end plate of rat tibialis anterior muscle demonstrated by reaction with alizarin red S administered intramuscularly *in vivo*. The muscle was stimulated by prior intraperitoneal injection of neostigmine methylsulfate. ×775.



FIGS 12-21

muscle through the sciatic nerve, or 5 min after intraperitoneal injection of 0.2 ml 1 mM neostigmine methyl sulfate, which produced generalized muscular fasciculations, a large quantity of calcium was demonstrated in the end plate by injection of alizarin red S into the muscle (Fig. 21). Calcium ions appeared as a red colored "lake" formed by reacting with the dye, which had the same localization and appearance as was produced by staining for bound metal ion or cholinesterase, but lacked the finer details of such staining. No localization of dye occurred without prior stimulation of the muscle electrically or by administration of neostigmine.

DISCUSSION

The motor end plates of most striated muscles are known to be located at the midpoint of the muscle fibers, and to be arranged linearly in the muscle, forming the terminal innervation band (2). However, the three-dimensional distribution of the end plates has not been well studied. This distribution was investigated in the rat tibialis anterior muscle, in order to enable solutions of metal ions to be injected more correctly into the end plate zone. The zone of motor end plates in the tibialis anterior muscle has the shape of a dome, the apex of which is directed proximally, probably because the superficial fibers terminate more distally at the tendon of insertion than the deeper, shorter fibers.

The procedure for determining the binding of metal ions differed in some respects from the original method of Sávay and Csillik (18, 19). Metal ions were dissolved in distilled water or buffer solution prior to injection, rather than in formalin plus urea or guanidine. Formalin is known to inhibit the affinity of tissues for metal ions, and urea and guanidine were found to be unnecessary. Once binding of metal ion had occurred, the frozen tissue sections could be fixed in formalin vapor. It was also found that the treatment of muscle sections with an aqueous solution of sodium sulfide (18, 19) caused alterations in tissue structure during the succeeding procedures. Such alteration was avoided by treating the sections with sodium sulfide dissolved in 70% ethanol or 0.2 N acetic acid, followed by aluminum potassium sulfate solution.

The motor end plates bound preferentially not only with lead ions in aqueous solution, but also with stannous, cadmium, zinc and cupric ions in aqueous solutions. Under the conditions of the experiment, cobaltous, ferrous and mercuric ions did not bind, but it is possible that this failure might be demonstrated by altering pH or other experimental conditions, as occurred with cadmium and zinc ions. Solutions of calcium, cadmium, barium and magnesium in 80% ethanol have also been demonstrated to bind to the end plate, but aqueous solutions did not (20). It is evident that the motor end plate has affinity to a variety of divalent metal cations.

When muscle was stimulated through the nerve or by administration of neostigmine, the intramuscular injection of alizarin red S resulted in "lake" formation at the neuromuscular junction, as previously reported by Csillik and Sávay (7). Injection of the dye *in vivo* yielded a much finer pink colored lake than was attained by these authors, who treated excised muscle with the dye. Since calcium is the only substance known to be present in the body in sufficient concentration to form a lake with alizarin red S, the reaction is attributed to release of this ion from the end plate as a result of nerve stimulation or neostigmine administration. Other ions which can form a lake with the dye, such as aluminum, cadmium and lead, are normally not present in sufficient concentration to do so. Acetylcholine, sodium, potassium and magnesium, which participate in excitation, do not form lakes with the dye. Since calcium appears to be concentrated in the end plate in bound form, from which it is released following muscle excitation, the binding of other divalent cations with the end plate may result from replacement of calcium by these ions, which have lower ionization tendencies. The affinity of lead, stannous, cadmium and zinc ions for the end plate appeared to vary inversely with their ionization tendencies.

The distribution and appearance of end plates stained by sulfides of lead, tin, cadmium, zinc or copper were the same as those of end plates visualized by staining for cholinesterase activity or with Janus green B (11), and differed from those visualized by methylene blue staining. The subneural apparatus of Couteaux (3), including the secondary synaptic clefts, was visualized, indicating that these divalent metal ions bind to the postsynaptic membrane. Electron microscopic studies have confirmed the binding of lead to the postsynaptic membrane (6), primarily to the cold (Lehner, cited in reference 4) or sarcoplasmic columns (24). The persistence of metal-binding and cholinesterase activities of the end plate for

several weeks after denervation supports their postsynaptic localization as the electron microscopic studies demonstrated degeneration of axon terminal without changes in the postsynaptic components of the end plate following denervation (1). While the sites of metal-binding and cholinesterase activity are very close together, they are apparently distinct, since freezing or formalin fixation of muscles completely inhibited the binding of metal ions, but did not greatly affect cholinesterase activity. The effect of freezing and thawing on lipoprotein configuration (9) and polarization microscopy studies of lead binding (4) have suggested that lipoprotein may be the molecular site of binding of divalent metal ions to the end plate. There is some evidence that the neuromuscular blocking action of calcium (5) and uranyl (12) ions may be due to their binding to the phospholipid moiety of the postsynaptic membrane. However, there are undoubtedly other molecular sites of metal binding, including sulfhydryl groups (10) and ribonucleoprotein (22).

While the site of binding of divalent metal ions to the postsynaptic membrane appears to be adjacent to, but distinct from cholinesterase, its relation to the acetylcholine receptor is less clear. The prior administration of acetylcholine, *d*-tubocurarine, neostigmine or diisopropyl fluorophosphate prevented the binding of cadmium and zinc ions, but did not detectably affect the manner binding of lead and stannous ions, which is less influenced by changes in the chemical environment, including pH. Prior administration of acetylcholine plus physostigmine has been reported to alter the binding of lead to the postsynaptic membrane, as indicated by polarization microscopy (4). These observations suggest that study of the metal-binding sites may eventually provide information concerning the acetylcholine receptor, although the problem is complicated by the high concentration of compounds used in these studies, the partially presynaptic actions of neostigmine (16) and perhaps *d*-tubocurarine (21) and similarities between the properties of the acetylcholine receptor and of the anionic site of cholinesterase (25).

A number of metal ions have been found to affect muscle contraction (17), but their effect on neuromuscular transmission has been relatively little studied. Calcium decreases the permeability of the postsynaptic membrane (23) and inhibits depolarization of the membrane by carbamoylcholine, probably by competing for the acetylcholine receptor site (15). Uranyl ions were also found to block neuromuscular transmission (12), and complex ions of neutral chelating agents with ferrous, nickel, cobalt, ruthenium or osmium ions have been reported to have neuromuscular blocking activity (8). However, the physiologic significance of the high affinity of the postsynaptic membrane for divalent metal cations remains to be elucidated.

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National Academy of Sciences, 1972

Annual Poundage Reported Per Substance by NAS and FEMA User Firms

National Academy of Sciences Preliminary Data Tables, Washington, D. C.

(Pertinent section included in monograph text.)

ANALYSIS OF FRUIT JUICE BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

I.—The determination of iron and tin in canned juice

By W. J. PRICE and J. T. H. ROOS

Atomic absorption spectrophotometry has been used to compare the concentrations of iron and tin in canned fruit juice with those in juice not stored in cans. No significant difference was found in the level of iron, but the amount of tin was considerably higher in canned samples. The determination is rapid, and no ashing procedure is required.

Introduction

The determination of traces of contaminants leached from containers in which foodstuffs are stored is of considerable public importance. Foodstuffs which are stored in cans, especially those such as fruit juices which contain a certain residual acidity, are liable to dissolve traces of tin, iron and lead from the container. Iron may be determined readily with o-phenanthroline[1] or similar reagents,[2] but the determination of lead with dithizone[3] and tin with, for example, dithiol reagent[4] are tedious and susceptible to a variety of interferences. For this reason, the possibility of using atomic absorption spectrophotometry for the determination of iron, lead and tin was investigated. However, it soon became apparent that the concentration of lead in the samples analysed was too low for direct determination. Since the atomic absorption technique is both rapid and relatively free from interference, attention was focused on developing a method yielding equally rapid sample pre-treatment.

The determination of tin by atomic absorption spectrophotometry has been described by several authors, most of them have used air–hydrogen flames. The sensitivity of tin absorption in the air–acetylene flame was found by Gatehouse & Willis[5] to be about 5 ppm. Allan[6] used the air–hydrogen flame for increasing tin sensitivity. Amos & Willis[7] used the hotter nitrous oxide–acetylene flame for tin determination and found that the sensitivity was intermediate between that of the air–hydrogen and air–acetylene flames. Amos[8] has recently suggested that interferences which occur in the air–hydrogen flame may be eliminated when the nitrous oxide–acetylene flame is used.

The atomic absorption determination of iron has been extensively investigated and is reported[9] to be virtually interference-free. The determination of iron in plant material and beverages[10] has been described.

Experimental

Apparatus and reagents

A Unicam SP90 Atomic Absorption Spectrophotometer complete with triple lamp turret and nitrous oxide control unit was used throughout this study. The air supply was a Unicam SP93 Air Compressor, and recorder traces were obtained on a Unicam SP22 Recorder. Hollow cathode lamps were supplied by Pye Unicam Ltd.

A Gallin Christ 'Universal Junior III' centrifuge (supplied by Gallin & George Ltd., London) was used for removal of suspended matter.

Iron stock solution, 1,000 ppm: 1·0 g of high purity iron (as S 270 : 1) was dissolved in 20 ml of hydrochloric acid (sp. gr. 1·16) and 5 ml of 100 vol. hydrogen peroxide, the solution boiled to remove excess peroxide and diluted to 1 litre in a volumetric flask.

Tin stock solution, 1,000 ppm: 1·0 g of Specpure tin metal was dissolved in 200 ml of hydrochloric acid (sp. gr. 1·16) and diluted to 1 litre in a volumetric flask. This solution was found to be stable for at least six months.

Nitric acid (sp. gr. 1·43), hydrochloric acid (sp. gr. 1·16) and perchloric acid (60% wt. vol.) were all of analytical reagent grade quality and were used without further purification.

Solutions used in investigating possible interferences were prepared from analytical reagent grade chemicals unless otherwise specified.

All solutions were made up in de-ionised water.

Investigation of instrumental conditions

Iron

With a monochromator slit of 0·1 mm (corresponding to a spectrum bandwidth of 0·6 nm) calibration graphs for iron (0–80 ppm) were obtained at different acetylene flow rates between 900 and 1,500 ml/min and different heights (between 0·5 and 1·5 cm) of the light path above the burner top ('burner height' or 'observation height'). The air flow rate was fixed at 5 litre/min. Best sensitivity was obtained with the resonance line at 248·3 nm using an acetylene flow rate of 1,200 ml/min and burner heights of 0·8 to 1·0 cm. The effect of varying the slit width between 0·05 mm and 0·15 mm was also investigated. Predictably, best sensitivity was obtained with the smallest slit width, and sensitivity fell off slightly with wider slits. The sensitivity in a nitrous oxide–acetylene flame was lower than that achieved in an air–acetylene flame by a factor of 2.

Tin

Using a nitrous oxide flow rate of 5 litre/min, the effects of variations in the acetylene flow rate (3,000–5,000 ml/min) and burner height (0·5–1·5 cm) on the tin absorption were investigated at the most sensitive tin wavelength (286·3 nm). Best sensitivity was found when the acetylene flow rate was greater than 4,000 ml/min, with a burner height of 0·5 cm; increasing the acetylene flow rate above 4,000 ml/min produced a negligible increase in sensitivity. The effect of slit width was similar to that for iron.

In an air–acetylene flame, even a highly fuel-rich flame, the sensitivity was lower by a factor of 4 compared with that in nitrous oxide–acetylene, and was not further investigated.

Recommended operating conditions for the determination of both tin and iron are summarised in Table I.

Interferences

Constituents of fruit juices likely to interfere in the estimation of iron and/or tin are potassium ions, citric acid, phosphate ions and sugar. Investigation showed that, of these, both citric acid and sugar interfered seriously in the determination of iron, whilst the determination of tin in the nitrous oxide–acetylene flame was free from interference. The depressive effect of citric acid and sugar on the iron absorption was more noticeable in a fuel-rich air–acetylene flame than in a lean flame; in either case it could be completely removed by the addition of phosphate although very much more phosphate was required when using a fuel-rich flame. Typically, using a lean (therefore hot) flame, the addition of 60 ppm PO_4^{3-} completely eliminated the depressive effect, on 5 ppm of iron, of 10,000 ppm (1% wt./vol.) citric acid. The effect of citric acid could also be eliminated by using a propane–nitrous oxide or an acetylene–nitrous oxide flame, but with a sacrifice of some sensitivity for iron. Because of the necessity to work under conditions of maximum iron sensitivity, and because fruit juices normally contain at least 200 ppm of phosphate ion which is sufficient to overcome the interference from citric acid, a lean air–acetylene flame was used for the determination of iron. The effects of added substances on the iron absorption are summarised in Table II.

Development of the method

Dry ashing of samples of fruit juice was found to be unsatisfactory. Not only was prior evaporation of the sample required, but heating at 500–550° for four hours and longer was insufficient to ash the samples completely. Wet ashing with a mixture of nitric acid and perchloric acid proceeded smoothly and efficiently, but much of the tin in the sample was precipitated as hydrated stannic oxide under these conditions.

The possibility of using a simple dilution of the juice for direct determination of iron and tin was investigated. In order to ascertain the effect of viscosity on such a method known quantities of iron were added to successive dilutions of natural orange juice. These were aspirated in the atomic absorption spectrophotometer, and the apparent concentration of iron was determined for each solution. In order to eliminate possible interferences, these determinations were performed using a nitrous oxide–acetylene flame. The results are shown in Table III.

Procedure

A 20 ml portion of the sample was transferred to a 100 ml volumetric flask, 10 ml hydrochloric acid was added and the sample was diluted to the mark with water. After thorough mixing a portion of this solution was centrifuged (or, if conveniently, filtered) for analysis. Calibration standards were prepared for iron (0–10 ppm) and tin (0–50 ppm) in 10% (wt./vol.) hydrochloric acid. Using the instrument settings recommended in Table I, the standard solutions followed by the sample solutions were aspirated.

Accuracy and reproducibility

The accuracy of the proposed method was assessed by analysing samples to which known quantities of iron and tin had been added. The recoveries obtained are given in Table IV. The reproducibilities (expressed as standard deviations) for both iron and tin were calculated on the results of eleven replicate analyses: eleven 20 ml portions of a sample of pineapple juice were treated as outlined under 'Procedure' and aspirated for iron and tin. The results are summarised in Table V. The smallest concentrations of iron and tin in undiluted juice which could be detected by the proposed procedure were 0·5 ppm and 2·5 ppm respectively. These figures are equivalent to absolute detection limits of 0·1 ppm and 0·5 ppm respectively, but the values will vary somewhat from one hollow cathode lamp to another.

Results and Discussion

The concentrations of iron and tin were determined in bottled orange juice (fresh, bottled and canned) and pineapple juice (bottled and canned). Each sample was analysed in duplicate according to the proposed method. The results are given in Table VI.

TABLE I

Recommended operating conditions for iron and tin

	Iron	Tin
Wavelength	248·3 nm	286·3 nm
Slit width	0·08–0·1 mm	0·08–0·1 mm
Spectrum bandwidth	0·5–0·6 nm	0·7–0·9 nm
Burner height	0·8–1·0 cm	0·5 cm
Oxidant	Air, 5 l/min	N_2O, 5 l/min
Fuel	Acetylene	Acetylene
Fuel flow rate	1200 ml/min	4000 ml/min
Scale expansion	2–3 times	none
Calibration standards	0–10 ppm	0–50 ppm

TABLE II

Effect of added substances on iron* absorption

Substance	Concentration	Absorbance	Error, %
Nil	—	0·171**	—
Citric acid	50 ppm	0·140**	18
Citric acid	100 ppm	0·118**	31
Sugar	4%	0·164**	4
Nil	—	0·175***	—
Citric acid	50 ppm	0·130***	26
Citric acid	100 ppm	0·088***	50
Sugar	4%	0·142***	19

*30 ppm iron in each case
**lean air–acetylene flame
***fuel-rich air–acetylene flame

TABLE III

Effect of dilution of fruit juice on iron response

Solution, ml juice/100 ml	Dilution factor	Iron present, ppm	Apparent iron concentration, ppm	Error, %
0	∞	200	200	—
5	20	200	199	0·5
10	10	200	199	0·5
20	5	200	198	1·0
40	2·5	200	194	3·0
80	1·25	200	187	6·5

TABLE IV

Recoveries of added iron and tin

Iron, ppm					Tin, ppm				
Added	Recovered				Added	Recovered			
	Sample A		Sample B			Sample A		Sample B	
0	3·6,	3·7	5·0,	5·1	0	47·0,	47·0	37·7,	34·3
3	6·4, 6·4	6·3	8·1,	8·0	10	56·0,	58·5	46·3,	46·0
6	9·5,	9·5	11·4,	11·3	20	66·0,	66·0	58·5,	58·5

TABLE V

Reproducibility of the method

Sample No.	Iron found, ppm	Tin found, ppm
[illegible]	24·0	144
[illegible]	24·0	146
[illegible]	23·8	144
[illegible]	23·8	148
[illegible]	23·6	152
[illegible]	23·6	148
[illegible]	23·8	142
[illegible]	23·6	150
[illegible]	23·2	150
[illegible]	23·8	154
[illegible]	23·6	148
Standard deviation	0·21 ppm or 0·9%	3·5 ppm or 2·3%

TABLE VI

Iron and tin in fruit juice

Brand	Description	Iron, ppm	Tin, ppm
—	Fresh orange juice	~0·5, ~0·5	7·5, 7·5
A	Bottled orange juice	2·5, 2·5	30, 25
B	Bottled orange juice	2·2, 2·0	45, 50
C	Bottled pineapple juice	15·5, 15	45, 50
D	Canned orange juice	2·5, 2·5	65, 60
E	Canned orange juice	0·5, 0·5	115, 115
F	Canned orange juice	2·5, 2·5	115, 120
G	Canned pineapple juice	17·5, 17·5	130, 135

As can be seen, very little difference exists in the concentration of iron in canned and bottled juice. However, the concentration of tin appears to be significantly different, with canned juices having predictably a much higher concentration of tin.

An attempt was also made to determine lead in the same solutions, but the level of lead present (if any) was too low to be detected. This indicates a lead content of less than 0·5 ppm in the undiluted samples.

Because of the high sugar content of juice, undiluted samples have a high viscosity. This results in a reduced uptake rate in an atomic absorption spectrophotometer, consequently giving rise to low results. With increasing dilution this effect becomes less and less important, and a 1:5 dilution of the juice is sufficient to minimise the error incurred. A 1:5 dilution is also adequate to prevent blocking of the nitrous oxide burner by the sample solutions. Addition of 1 ml of 1% (by vol.) phosphoric acid to the diluted samples prior to analysis is recommended as a precaution against citrate interference, especially for samples low in phosphate or high in iron.

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Papers impregnated with the following organic and inorganic adsorbents were also used: ammonium oxalate, ammonium carbonate, glucose, naphthalene, benzoic acid and sodium chloride.

The successful separations are given in Tables I, II and III. The systems that gave the best results are marked with an asterisk.

In order to find out whether the separations can be of use in qualitative analysis $M/5$ test solutions of $SnCl_2$ and $SbCl_3$ were mixed in different ratios and separated by ascending chromatography using a mixture of anisole and formic acid (1:1) as the developer. In all cases good separations were obtained. The papers were conditioned for 1 hour prior to chromatography. The results are summarized in Table IV.

TABLE IV

SEPARATION OF MIXTURES OF Sn^{2+} AND Sb^{3+} OF VARYING COMPOSITION

Ratio of $SnCl_2$:$SbCl_3$	R_F		Time of development h
	Sn^{2+}	Sb^{3+}	
2:1	0.18	0.52	1
3:1	0.19	0.51	1
4:1	0.22	0.53	1
1:2	0.10	0.48	1
1:3	0.17	0.61	1
1:4	0.17	0.52	1

The test solutions were diluted to $M/50$ and $M/100$. When these solutions were spotted and developed with anisole–formic acid (1:1), after conditioning for one hour, good separations were obtained. The results are summarised in Table V.

TABLE V

SEPARATION OF Sn^{2+} AND Sb^{3+} AT HIGHER DILUTIONS (ASCENDING TECHNIQUE)

Concn. of test solution	R_F		Time of development h
	Sn^{2+}	Sb^{3+}	
$M/50$	0.06	0.38	1
$M/100$	0.01	0.36	1

The zones of Sn^{2+} were always compact, well defined and well separated, while the zones of Sb^{3+} were sometimes a little diffuse.

On repeating the separation reported by STEFANOVIĆ *et al.*[4] it was found that besides being time-consuming (21 hours) their method is not reproducible. Similarly the separation advocated by HARASAWA[3] fails under the conditions specified. It is possible that in our separations formic acid plays a beneficial role by preventing the oxidation of Sn^{2+} and Sb^{3+} to higher valence states.

Butyric acid commercial 50% (E. Merck) and anisole, free from alcohol (Naarden), were used. All the other chemicals were of reagent grade, either from E. Merck, or British Drug Houses. Schleicher & Schüll No. 2043a sheets were used for ascending and descending chromatography. Whatman No. 1 filter paper circles (diameter 12.5

cm) were used with the Kaweran apparatus. The $M/5$ test solutions of $SnCl_2$ and $SbCl_3$ for the experiments given in Tables I–IV were prepared as follows:

1.89 g of $SnCl_2$ and 2.28 g of $SbCl_3$ crystals were dissolved in 15 ml of conc. HCl separately and the solutions were then diluted to 50 ml with distilled water. The $M/50$ and $M/100$ test solutions were prepared by subsequent dilution with 30% HCl. Two spots (approx. 0.0014 ml) of each of the two test solutions were applied to the test paper with a thin glass capillary. The cations were detected by hydrogen sulphide gas. The room temperature was 26° ± 4°.

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A simple apparatus for keeping the final pressure in gas chromatographs constant

In the chromatographic analysis of gases with the aid of katharometer detectors both the peak height and the retention time depend on the flow rate of the carrier gas; the peak height moreover depends on the pressure in the detector cell. The factors which affect the flow rate of the gas are the pressure before the column, the resistance of the column, and the pressure after the column. The pressure before the column and the resistance of the column can be easily kept constant by using suitable regulating valves and by thermostating the column. The pressure after the column, however, depends on the barometric height. In serial tests, in which the calibration curve is not checked after each measurement, the change in barometric height over a few hours or days may cause considerable deviations from the calibration curve; according to our experience, the peak height varies by about 0.1% upon 1 mm change in barometric height. A diagram of a simple apparatus for keeping the pressure after the column constant is shown in Fig. 1.

The exit of the detector cell 1 is provided with a needle valve 2. A differential pressure gauge 3, which measures the pressure difference between the points 1 and 4 is fitted between the cell and the valve. 4 is a glass bulb with a capacity of approx

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Photometric Method for the Determination of Small Quantities of Tin Using Phenylfluorone

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Photometric methods for the estimation of tin are usually complicated by the need to separate tin from substances such as phosphate, fluoride, or metals that interfere with the color development. Distillation of tin as the volatile halide is often used to accomplish the separation, but this requires much apparatus and is very time-consuming. Kraus and Nelson (*Symposium on Ion Exchange and Chromatography in Analytical Chemistry* ["Publications of the American Society for Testing Materials," No. 195], p. 27, 1956) have shown that many metals, including tin, form *anionic* complexes that are adsorbed on suitable resins in the presence of HCl. There remain the difficulties of eluting the other interfering anions and of recovering the tin quantitatively. Ariel and Kirowa (*Talanta*, 8:214, 1961) have recently overcome the problem of the elution of tin by using 6 N NaOH to convert the stannic complex into a soluble stannate. In this way the complete recovery of 1 mg. tin was obtained under their particular conditions.

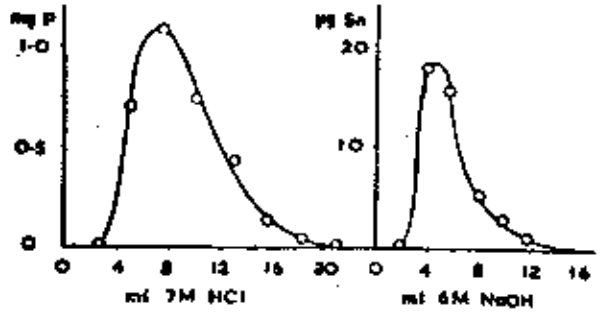

FIG. 1.—Elution of phosphate and tin from resin.* The sample consisted of 18 mg. hydroxyapatite containing 40 µg. tin; 41.2 µg. tin was recovered. In this trial experiment, the estimation of tin in 2.0-ml. fractions of the eluate necessitated modifications of the procedure described in the text.

The following method was developed to determine the uptake of tin by synthetic hydroxyapatite and tooth surfaces. With minor modifications, this method can be used for the determination of tin in many biological samples and in stannous fluoride dentifrices.

A weighed sample was dissolved in 4.0 ml. of 7 M HCl, and 1.0 ml. of 3 per cent H_2O_2 (w/v) was added. Aliquots containing 4–40 µg. tin were transferred to the resin column. A resin* of the strongly basic, cross-linked polystyrene type was used in the acetate form. The column dimensions were 5.5 cm. by 0.8 cm., and the average flow rate was 1.4 ml/minute. Pretreatment of the resin with 7 M HCl and 6 M NaOH was found necessary; this was continued until the resin blank gave a minimal reading. Regeneration of the resin was carried out in bulk with 3 per cent acetic acid. The columns were washed with 18.0 ml. 7 M HCl to elute phosphate and fluoride (Fig. 1). Stannic tin remains completely adsorbed on the resin at this molarity of HCl. Tin was eluted with 12.0 ml. 6 M NaOH, and the eluate was acidified (in ice-bath) with 7.2 ml. 11 M HCl and diluted to 50.0 ml. with 1 M HCl.

The photometric method of Bennett and Smith (*Anal. Chem.*, 31:1441, 1959) was used, but the addition of H_2O_2 was omitted. To 10.0 ml. of the eluate was added about 6.5 ml. acetate buffer, and the pH was thus brought to pH 3.5 ±0.1. Deionized water was added to the 20.0 ml. mark, followed by 3.0 ml. phenylfluorone (25 µg/ml). After 10 minutes, the optical density was read at 540 mµ in glass cells with 4 cm. light paths. In addition to the unknowns, reagent blanks and standard solutions were run through the columns, and a standard curve was thus plotted. Over the range 4–40 µg., the recovery of tin was 97–103 per cent.

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* De-Acidite FF, Permutit Company, Ltd.

TOXICOLOGY AND APPLIED PHARMACOLOGY 13, 332–338 (1968)

The Effect of Stannous Fluoride and Stannous Chloride on Inflammation

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The Effect of Stannous Fluoride and Stannous Chloride on Inflammation. STONE, ORVILLE J., and WILLIS, CAROLYN J. (1968). *Toxicol. Appl. Pharmacol.* **13**, 332–338. Scratches were made to the depth of the upper dermis on the abdomen of rabbits. The scratches were covered by patch tests for 18 hours with solutions of stannous fluoride or stannous chloride. Both these substances produced a destructive reaction with intraepidermal polymorphonuclear leukocyte pustules occurring on each side of the scratch. Stannous fluoride was destructive at lower concentrations than stannous chloride. When these substances were patch tested over non-traumatized tissue, no tissue damage occurred. It is suggested that stannous fluoride and stannous chloride may interfere with the enzymes of inflammation. Salts of fluoride, iodide, nickel, arsenic, and mercury are known occasionally to produce a pustular reaction when used to patch test humans.

Persons patch tested with metal salts or halogens sometimes develop pustules in the test site (Fisher *et al.*, 1959; Fisher, 1967). This can occur with a number of metal salts, but it is more common with nickel, mercury, and arsenic. The reaction occurs also with iodides but is more common and severe when it occurs with fluorides. The reaction does not represent an irritant or an allergic reaction, and it is not readily reproducible on patients. Recently it was found that preexisting inflammation was present at sites that develop into a pustular patch test, and it was suggested that the substances that produce the pustular patch test interfere with the enzymes of inflammation (Stone and Johnson, 1967). An animal model has been developed to study the effect of these chemicals on inflammation (Stone and Willis, 1967a, 1968b). When 0.25% ammonium fluoride or 0.5% sodium fluoride in water are placed as a patch test over a scratch on the rabbit's abdomen, a row of intraepidermal pustules develops on each side of the scratch by 18 hours. The purpose of this paper is to evaluate the effect of stannous fluoride and stannous chloride on inflammation.

METHODS

Part 1

Sixteen white rabbits (6–8 pounds) were clipped over the abdomen 2 days before the experiment. The animals were scratched in the shape of an "X" at each test site. The scratch was deep enough to break through the epidermis, but little or no bleeding occurred. The suspensions of stannous fluoride and stannous chloride were prepared

the day of the study and are reported as the percentage of the salt in distilled ter. The stannous chloride test animals were tested with concentrations of 2%, 1% 0.5%, 0.25%, and 2 water control sites. The stannous fluoride test animals were ted with concentrations of 0.5%, 0.25%, 0.1%, 0.05%, and 2 water control sites. ht animals were used to test each substance. Three-tenths of a milliliter of each lution was placed on a piece of gauze and applied to a scratch. The gauze was ered with plastic and waterproof tape. The test sites were then protected by cover- them with gauze and taping around the animal's body. At the end of 18 hours, the tches were removed.

n 2

ten rabbits were prepared as in Part 1 except that they were not scratched. Five e patch tested over intact skin with stannous chloride 1% at two test sites and ter patches over two control sites. The other five were tested by the same method ept that stannous fluoride 0.25% was used instead of stannous chloride. After 18 hours, the patches were removed.

. 1. Scratch on rabbit's abdomen 18 hours after patch testing with 0.25% stannous fluoride.

RESULTS

In Part I the control sites consistently had no pustules. The sites tested with stannous chloride 2% and 1% always had a continuous row of pustules on each side of the scratch, and pustules never occurred at lower concentrations. The 0.5% and 0.25% stannous fluoride sites always had a row of pustules (Fig. 1) on each side of the scratch, but the 0.1% and 0.05% sites were consistently negative for pustules. Multiple biopsies were taken from the sites tested with stannous chloride 1% and 0.5%

FIG. 2. Stannous fluoride 0.25%. The epidermis is intact on the right, and only the stratum corneum is intact on the left. Hematoxylin and eosin, ×180.

and stannous fluoride 0.25% and 0.1%. The pustules were cultured on blood agar and in thioglycolate and were consistently sterile by these techniques.

The biopsy findings were similar for both stannous chloride 1% and stannous fluoride 0.25%. Both lesions included an intraepidermal polymorphonuclear pustule with complete destruction of the epidermis but with the stratum corneum intact (Fig. 2). The upper dermis showed fragmentation and edema of the collagen with a marked infiltrate of polymorphonuclear leukocytes and mononuclear cells (Fig. 3)

here was marked fragmentation of cells with cellular debris present in large amounts throughout the lesion. The biopsies of the stannous chloride 0.5% and the stannous fluoride 0.1% sites showed a cellular infiltrate in the area of the scratch but the epidermis on the edges of the scratch was not invaded by an infiltrate (Fig. 4). The infiltrate was largely polymorphonuclear leukocytes with a few mononuclear cells. The inflammatory infiltrate around dermal vessels was increased. Control site tissue

Fig. 3. Stannous fluoride 0.25%. Destructive infiltrate of upper dermis showing cellular debris and destruction of collagen. Hematoxylin and eosin. ×360.

showed some areas of mild upper dermal edema, a mild infiltrate of mononuclear cells and very few polymorphonuclear leukocytes.

When stannous chloride 1% or stannous fluoride 0.25% is placed as a patch test on a scratch on the rabbit's abdomen for 18 hours, a destructive response occurs. A marked polymorphonuclear leukocyte reaction destroys the epidermis and the upper dermis. A row of pustules occurs along each side of a scratch. Sites that are patch tested with lower concentrations, stannous chloride 0.5% and stannous fluoride 0.1%, will not produce pustules but will still produce a polymorphonuclear

or necrosis at the site of all large pustules. Clinically, the fluoride pustule is the only pustular patch test lesion that results in scarring.

Douglas (1957) has reported 133 cases of stomatitis which he has attributed to fluoride dentifrices. He described the lesions as being superficially ulcerated with whitish exudate and vesicles. He noted that the reaction was more difficult to clear if it had been present for a long time and that "any little bump to the cheek or trauma of any nature would immediately cause the lesions to revert to the more severe ulcerative states."

More recently, reactions to fluoride dentifrices have been referred to as allergic reactions (Shea *et al.*, 1967). We question the advisability of referring to the reaction as allergic, because we believe that the fluorides are exaggerating preexisting edema or inflammation. In studies using the model described in this experiment, nickel sulfate produces intraepidermal pustules (Stone and Willis, 1968a). When hyaluronidase is infiltrated under the site before the scratch is made, the pustule does not occur. When normal saline is injected into the upper dermis and covered by a nickel sulfate patch test, the site of the wheal becomes a pustule, but the site of the needle penetration does not. We believe that the metals and halogens that produce pustule along the scratch are damaging the body's defenses against the mediators of inflammation and are therefore exaggerating the early cellular phase of inflammation.

We now know that the pustular patch test is a reaction pattern. Arsenic produces pustular patches only over sites of bacterial infection (Stone and Willis, 1968b), and this occurs because arsenic damages the production of the mediators of inflammation (Stone and Willis, 1968c). This suppresses the body's defense mechanisms. Sodium arsenate blocks the pustular response to sodium fluoride.

We do not know specifically what enzyme system or systems are being damaged by stannous chloride or stannous fluoride, but it is known that some metal salts are capable of inactivating kininases which remove the kinin mediators of inflammation (Erdos *et al.*, 1963). We do not feel that a simple toxic reaction is occurring, since many toxic chemicals have failed to produce this lesion. Iodides will not produce pustules when placed as a patch test over a scratch; however, they will increase inflammation locally over a site of bacterial injection. Iodide given systematically increases inflammation (Stone and Willis, 1967b).

Dentifrices are not made for prolonged contact with tissue; however, even brief exposure might influence preexisting oral pathology. Further clinical observations on the effects of fluoride dentifrices on oral inflammation are indicated.

In our previous work on the effect of simple chemicals on inflammation, we have emphasized that increasing inflammation could be either helpful or harmful, depending on a number of complex variables. Iodide is used to treat chronic granulomas by increasing the inflammatory responses. Arsenic suppresses early inflammation and exaggerates infectious processes. Inflammation in general is a highly beneficial response, and the effect of fluoride should be evaluated on each type of oral disorder.

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The first study on acid phosphatase in human semen [EC 3.1.3., phosphoric monoester hydrolase, semen] was conducted by Kutscher et al. [1] in 1935, in which they separated acid phosphatase from human urine. Subsequent investigation by other researchers such as those described in the literatures published by Walker et al. [2] and Schmidt [3] confirmed that this enzyme was produced by the prostate and found in human semen in large quantities.

The purification procedures of this enzyme have been developed by London et al. [4] and Bowman et al. [5], and more recently by Ostrowski et al. [5].

At the present, however, the substrate specificity of this enzyme is not clearly known. Many of the prior experiments used β-glycerophosphoric acid, phenyl-phosphoric acid, p-nitrophenyl phosphoric acid and other artificially synthesized materials which cannot be regarded as natural substrate. Unfortunately, experiments under this condition are unable to reveal the inherent properties of the enzyme. Lundquist [7,8] maintained that the semen contained choline-phosphoric acid secreted from the seminal sac, and this acid was the natural substrate of acid phosphatase. However, few experiments, including those by Lundquist [7,8], hydson et al. [9], and Stickland et al. [10], employed this acid as the substrate. Reviewing the literatures, the optimum pH for the hydrolysis of choline-phosphoric acid is not always in agreement, and the manner in which the hydrolysis takes place has not been fully

clarified. Moreover, in the event that the substances which could work as the receptor of phosphoric acid, such as fructose contained in the semen in a large quantity, react with choline-phosphoric acid as the substrate, their effects on the phosphatase activity must also be taken into consideration. The present investigation, motivated to solve these problems, led to a discovery that fructose accelerates the hydrolysis of choline-phosphoric acid. Such effect, however, depends greatly on the type of the synthetic choline-phosphoric acid sample used. In order to pursue the difference in the effect of fructose, chemical analysis was performed of a sample which gave positive reaction to fructose and a sample which exhibited no change in its hydrolytic process in the presence of fructose. As a result of this experiment, stannous ion contained in the choline-phosphoric acid sample was assumed to be responsible for the difference in the effect of fructose. The influence of metal ions on enzyme activity is within the rangeof valid enzymological analogy. Thus, the present experiment was designed to pursue the effect of tin on seminal acid phosphatase while comparing the enzymological and chemical properties of two types of choline-phosphoric acid samples.

EXPERIMENTAL PROCEDURES

1. PREPARATION OF ENZYME SOLUTIONS

1. COLLECTION OF HUMAN SEMEN AND PURIFICATION OF ENZYME

Of the seminal specimens of the patients who came to Kobe Medical College Hospital for semen test for male sterility, those within normal range were frozen and stored at below 0°C.

For purification, the frozen specimen was thawed, combined with 4 parts of 0.2 M acetate buffer solution (pH 5.5), stirred, left standing at 0°C for one hour, and precipitated with freezing centrifugal sedi-

mentator ($0^{o}C$; 10,000 rpm; 10 min), to remove part of sperms and proteins. The supernatant was subjected to dialysis with running water for 48 hours and with distilled water for 24 hours, then to centrifugation ($0^{o}C$; 10,000 rpm; 10 min), and the supernatant was used as enzyme solution. The above purification procedure elevated the specific activity of the enzyme toward protein by approximately 10 times (protein level, 1.5 mg/ml).

2. COLLECTION OF HUMAN PROSTATE AND PURIFICATION OF ENZYME

The prostates removed from the patients with benign prostatomegaly were immediately frozen and stored. The enzyme was purified according to the method used by Davidson et al. [11] (protein level, 0.6 mg/ml).

3. PURIFICATION OF ERYTHROCYTE ACID PHOSPHATASE [EC 3.1.3.]

The enzyme was purified from a human erythrocyte suspension solution supplied by a blood bank by the method proposed by Tuboi et al. [12] (protein level, 1.5 mg/ml).

4. PURIFICATION OF β-GLUCURONIDASE FROM THE PREPUTIAL GLAND OF WHITE RAT [EC 3.2.1.]

The preputial gland of white rat was combined with 0.25 M sucrose solution at 10% (w/v), and the mixture was homogenized and centrifugated at $0^{o}C$ and 100,000 g for one hour. The supernatant was 65%-saturated with ammonium sulfate, and centrifugated at low temperature and 17,000 g for 20 minutes. The residue was dissolved in physiological saline solution, and subjected to dialysis at $4^{o}C$ for 12 hours using the same physiological saline solution (protein level, 0.45 mg/ml).

Each enzyme solution was diluted with 0.02% egg-white albumin at use, and its concentration was so adjusted that 15 u mol of 60 u mol substrate would be hydrated under the conditions described in section V. In the case of β-glucuronidase, however, the solution was so diluted that the amount of substrate hydrated would be less than 50 mu mol

of 100 mu mol. To attain these substrate levels, the degree of dilution was 30-fold for both seminal acid phosphatase and β-glucuronidase, 25-fold for prostate acid phosphatase, 5-fold for erythrocyte acid phosphatase, and 500-fold for seminal fluid. The protein levels of these phosphatases were clearly higher than that of β-glucuronidase.

II. PREPARATION OF SUBSTRATES

Choline-phosphoric acid was prepared in the form of calcium salt by the method used by Plimmer et al. [13]. The calcium was eliminated quantitatively with sodium bromate. As described in Experimental Results, the sample which responds positively to fructose is referred to sample No. 1, and the sample without such tendency, as sample No. 2.

Phynyl-phosphoric acid was synthesized by the method employed by Jacobson et al. [14].

The synthesis of ethanol amine phosphoric acid followed the procedure formulated by Outhause [15], in which barium was quantitatively removed with sodium sulfate.

The β-glycerophosphoric acid used was a commercial product of Eastman and Kodack Co.

In the adjustment of the amount of substrates, the substrate concentration was adjusted at 60 u mol/ml in all cases after the pH value of thesubstrate solution was corrected to 5.0, 5.6, 6.4, and 7.6 for the pH ranges of 4.0 - 5.0, 5.3 - 5.9, 5.8 - 6.8, and 7.0 - 9.0, respectively, of the reaction solution.

The separation of β-phenylphthalene glucuronide and the preparation of the substrate solution followed the procedure employed by Talala et al. [16]. The concentration of the substrate solution was 1000 mu mol/ml.

III. SUGARS ANDOTHER ADDITIVES

The fructose used was a product of Merk Co., and other sugars, special-grade or 1st-grade products of Wako Junyaku Co. The sugars were subjected to specific rotation measurement and paper chromatography before use for the confirmation of their purity. The stannous chloride and stannic chloride, sodium fluoride and L-(+)-tartaric acid used were all products of Wako Junyaku.

IV. BUFFER SOLUTIONS

Three kinds of fubber solutions were used: 0.2 M acetic acid, 0.1 M maleic acid, 0.05 M veronal buffer solutions for the pH ranges of 4.0 - 5.9, 5.8 - 6.8, and 7.0 - 9.1, respectively. The use of a buffer solution containing hydroxide group was avoided to prevent the transfer of phosphoric acid.

V. REACTION CONDITIONS AND ENZYME ACTIVITY MEASUREMENT

1. MEASUREMENT OF PHOSPHATASE ACTIVITY

A mixture of 2.0 ml buffer solution, 1.0 ml additive or water, and 1.0 ml enzyme solution was preincubated for 5 min at 37°C, and, after 1.0 ml of substrate solution (60 u mol/ml) was added, the mixture was incubated for 15 min at 37°C. After the reaction was terminated with 1.0 ml of trichloroacetic acid, the solution was filtered. The amount of liberated phosphoric acid in a 1.0 ml portion of its filtrate and the amount of liberated choline in a 3.0 ml portion were measured. In the measurement of erythrocyte acid phosphatase activity, the substrate was β-glycerophosphoric acid and the reaction time, 1 hour.

2. QUANTITATIVE DETERMINATION OF PHOSPHORIC ACID

The quantitative determination of phosphoric acid followed Fiske-Subbarrow's procedure [17]. In this procedure, divalent tin inhibited

the coloration at over 2.0 mM of final concentration, but exerted no effect at less than 0.2 mM. Tetravalent tin showed no effect on the coloration at 2.0 mM. Liberated choline did not suppress the coloration reaction.

3. QUANTITATIVE DETERMINATION OF CHOLINE

The quantitative determination of choline followed Entenman's procedure [18].

4. MEASUREMENT OF β-GLYCURONIDASE ACTIVITY

The procedure used by Talalay et al. [16] was employed.

5. QUANTITATIVE DETERMINATION OF PROTEINS

The amount of proteins was measured by the procedure used by Lawry et al. [19]. The enzymic activity was expressed in terms of u mol/cup or mu mol/cup.

EXPERIMENTAL RESULTS

1. HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID BY SEMINAL ACID PHOSPHATASE

Choline-phosphoric acid (No. 1) is hydrolyzed favorably by seminal acid phosphatase. The results are compared with those of phenyl-phosphoric acid and β-glycerophosphoric acid normally used as substrate in Table 1. Dilute seminal fluid, purified seminal fluid, purified prostate extract solution, etc. were used as enzyme solution, but in any of these experiments, the speed of hydrolysis was higher in the order of phenyl-phosphoric acid - β-glycerophosphoric acid - choline-phosphoric acid, which denies the presence of special enzyme in choline-phosphoric acid. However, when dilute seminal fluid was used, choline-phosphoric acid exhibited a rate of hydrolysis slightly higher than other enzyme solutions. This seems to indicate that the semen contains a substance which works favorably only in the hydrolysis of choline.

II. EFFECTS OF FRUCTOSE ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID

Concentrating attention to fructose in semen among various factors which influence the hydrolysis of choline-phosphoric acid, the effect of fructose on choline-phosphoric acid was investigated. The experiment revealed that fructose stimulated the hydrolysis of choline-phosphoric acid (sample No. 1) to a marked degree (Table 2). Such an effect is manifest at its final concentration of 0.05 M or higher, particularly at over 0.25 M and at pH 5.0, as shown in Figure 1. Based on the experimental findings, subsequent experiments with fructose were performed under the conditions of 0.25 M concentration and pH 5.0. It was also noted that the effect of fructose decreased with higher concentration of enzyme.

The effect of fructose was suppressed in the experiment using choline-phosphoric acid sample No. 2 as the substrate, and the difference from the previous result was significant.

TABLE 1. HYDROLYSIS OF VARIOUSSUBSTRATES

Reaction time: 30 minutes

pH of reaction solution: pH 5.0 with phenyl-phosphoric acid and choline-phosphoric acid as substrate; pH 5.5 with β-glycerophosphoric acid as substrate.

Keys: a, enzyme; b, dilute seminal fluid; c, purified semen; d, extract of purified prostate; e, substrate; f, phenylphosphoric acid;

TABLE 1 - continued.

g, β-glycerophosphoric acid; h, choline-phosphoric acid; i, amount of liberated phosphoric acid; j, rate of hydrolysis.

TABLE 2. CONCENTRATION AND STIMULATORY EFFECT OF FRUCTOSE IN THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID SAMPLE NO. 1.

Enzyme solution: purified semen

Reaction condition: pH 5.0

The values in parentheses indicate the rates of acceleration.

Keys: a, concentration of fructose; b, amount of liberated phosphoric acid; c, amount of liberated choline.

TABLE 3. EFFECTS OF FRUCTOSE ON OTHER SUBSTRATES

Enzyme solution: purified semen

Reaction condition: pH 5.0

The values in the table indicate the amounts of liberated phosphoric acid.

Keys: a, concentration of fructose; b, substrate; c, phenylphosphoric acid; d, β-glycerophosphoric acid; e, ethanolamine phosphoric acid.

III. EFFECTS OF FRUCTOSE ON OTHER SUBSTRATES

With substrates other than choline phosphoric acid, fructose exhibited no positive effect on their hydrolysis, and even slightly inhibited the reaction in some instances (Table 3).

IV. DIFFERENCES BETWEEN TWO CHOLINE-PHOSPHORIC ACID SAMPLES

The difference in the behavior of fructose toward the two samples was unexpected. The following experiments were performed to clarify the difference between the two.

1. COMPARISON OF TWO SAMPLES BY pH-ACTIVITY CURVE

Experiments with and without fructose were performed using samples No. 1 and No. 2, and pH activity curves, shown in Figure 1 and 2, were obtained.

Without fructose, the optimum pH was 5.6 regardless of the enzyme solution, dilute semen or purified semen, the value being slightly to the acidic side from the pH 6.3 reported by Lundquist [8]. The experiment failed to confirm the presence of a special phosphatase which shows an optimum pH over 8.0, as was observed by Hudson et al. [9].

FIGURE 1. pH ACTIVITY-CURVE WITH SAMPLE NO. 1 AS SUBSTRATE.

Key: a, amount of liberated phosphoric acid.

Reaction conditions: pH 5.0; 37°C; 15 min.

—·—·— : 150-fold diluted semen; without fructose

------ :purified semen; without fructose

FIGURE 1 - continued.

——— : purified semen + 0.25 M fructose

—o— : acetate buffer

—Δ— : maleate buffer

—x— : veronal buffer

FIGURE 2. pH-ACTIVITY CURVES WITH CHOLINE-PHOSPHORIC ACID SAMPLE NO. 2 AS SUBSTRATE.

(Key and notes, same as Figure 1)

The experiments revealed three major differences between the two samples. First of all, when 0.25 M fructose was added, the hydrolysis of sample No. 1 was clearly accelerated within a certain acidic pH range, whereas such tendency was not observed in the hydrolysis of sample No. 2. Secondly, in the hydrolysis of sample No. 1, the addition of fructose caused a shift of optimum pH from 5.6 to 5.0. The third difference is the fact that sample No. 2 has a rate of hydrolysis approximately 3 times higher than that of sample No. 1. The measurement of liberated choline, performed at the same time, revealed that the substance liberated at the same gram molecule in both experiment, providing no evidence of the phosphoric acid contained in choline-phosphoric acid being transferred to

fructose during the hydrolysis by seminal acid phosphatase.

2. EFFECTS OF OTHER SUGARS ON TWO CHOLINE-PHOSPHORIC ACID SAMPLES

The effects of sugars other than fructose on the two samples were investigated and the results are listed in Table 4.

TABLE 4. EFFECTS OF OTHER SUGARS ON THE HYDROLYSIS OF TWO SAMPLES

Keys: a, sugar; b, D-fructose; c, D-dextrose; d, D-mannose; e, D-galactose; f, D-glucosamine; g, N-acetyl-D-glucosamine; h, D-sodium glucuronate; i, L-arabinose; j, D-arabinose; k, D-ribose; l, D-xylose; m, sucrose; n, lactose; o, malt sugar;p, dextrin ; q, adenosine; r, comparative effect; s, Sample No. 1; t, Sample No. 2.

Notes: The degree of acceleration of the hydrolysis of Sample No. 2 by other sugars was comparedwith that of Sample No. 1 by fructose set at +100%.

*1. The galactose recrystallized from glacial acetic acid showed notably reduced acceleration effect, with a relative value of +11.7. Further investigation must be made on this value.

*2. The concentration of dextrinewas same as 0.25 M dextrose.

*3. The concentration of adenosine was 0.05 M, and its effect was compared with that of 0.05 M fructose.

Glucosamine, D-ribose, D-galactose, lactose, and mannose also stimulated the hydrolysis of No. 1, but none of the sugars in this experiment influenced the hydrolysis of sample No. 2.

3. CHEMICAL ANALYSIS OF TWO CHOLINE-PHOSPHORIC ACID SAMPLES

Presuming that the composition and structure of the s mple were responsible for the enzymological difference, chemical analysis of the two samples were performed. The elementary analysis revealed that both samples were pure calcium chloride of choline phosphoric acid. In the spectral analysis of their infra-red absorption spectra, the absorption bands of both samples exhibited exactly the same wave pattern and degree of absorption. A part of the results of the emission spectrum analysis is shown in Figure 3.

FIGURE 3. EMISSION SPECTRA OF TWO CHOLINE-PHOSPHORIC ACID SAMPLES

Note: A part of spectral analysis.

Keys: a, wavelength; b, sample No. 1; c, sample No. 2.

The spectra show traces of Cu, Al, Mg, Si, and Fe in addition to Ca and P. What must be noted here is the fact that a trace amount of Sn is contained in sample No. 1 but not in sample No. 2.

Assuming that the difference in the effect of fructose on the hydrolysis of the two samples was caused by tin, the effects of tin and fructose on the hydrolysis of choline-phosphoric acid by seminal acid phosphatase was investigated.

V. INHIBITORY EFFECT OF STANNOUS ION ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID, AND THE EFFECT OF FRUCTOSE.

1. EFFECTS OF $SnCl_2$ AND $SnCl_4$ ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID.

As shown in Table 5, $SnCl_2$ reduces the hydrolytic capacity of seminal acid phosphatase, but the presence of fructose at the final concentration of 0.25 M suppressed the inhibitory effect of $SnCl_2$ almost completely. The degree to which the inhibitory effect of 0.2 mM $SnCl_2$ was suppressed by fructose was almost equal to the degree to which the hydrolysis of sample No. 1 was promoted by fructose (Figure 2).

Unlike $SnCl_2$, $SnCl_4$ failed to exhibit its inhibitory action at concentrations under 2.0 mM. However, it must be noted that its inhibitory action, once attained at effective concentrations, could not be suppressed by fructose.

TABLE 5. EFFECTS OF $SnCl_2$ AND $SnCl_4$ ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID.

Notes: Reaction condition - pH 5.0.

The values in the table indicate the amounts of liberated phosphoric acid.

Keys: a, concentration of tin; b, additive; c, without fructose; d, 0.25 M fructose

2. pH-ACTIVITY CURVES OF THE HYDROLYTIC ENZYME OF CHOLINE-PHOSPHORIC ACID IN THE PRESENCE OF $SnCl_2$ AND FRUCTOSE

Figure 4 shows the pH-activity curves of the hydrolysis of sample No. 2 in the presence of $SnCl_2$ and fructose. The figure clearly indicates that the influence of these additives. The optimum pH was 5.5 without addition in the medium, between 5.6 and 5.75 with 0.2 mM $SnCl_2$, and 5.0 with 0.2 M $SnCl_2$ and 0.25 M fructose. Thus, the shift of the optimum pH exhibited the tendency similar to that when fructose was added at the hydrolysis of sample No. 1 (See Figure 2).

FIGURE 4. pH-ACTIVITY CURVES OF THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID SAMPLE NO. 2 IN THE PRESENCE OF FRUCTOSE AND $SnCl_2$.

Key: a, amount of phosphoric acid

Reaction conditions: pH 5.0; 37°C; 15 min.

------ :without additive.

—·—·— : 0.2 mM $SnCl_2$

———— : 0.2 mM $SnCl_2$ + 0.25 M fructose

—o— : acetate buffer

—Δ— : maleate buffer

—x— :veronal buffer.

The most significant difference revealed by this experiment is the fact that the hydrolysis of No. 2 in the presence of $SnCl_2$ was accelerated

to a marked degree when the buffer solution was changed from acetate buffer to maleate buffer, whereas that of sample No. 1 was notably inhibited by maleate buffer. The reason for this phenomenon has not been clarified.

VI. INHIBITORY EFFECT OF $SnCl_2$ ON OTHER SUBSTRATES

An experiment was performed to determine whether or not the same reaction be expected from other substrates such as β-glycerophosphoric acid or phenylphosphoric acid, and the results are shown in Table 6.

As the table shows, the effects of $SnCl_2$ and fructose on the hydrolysis of these substrates were basically the same, indicating that such reaction is not specific to choline-phosphoric acid.

TABLE 6. INHIBITORY EFFECTS OF $SnCl_2$ ON THE HYDROLYSIS OF OTHER SUBSTRATES, AND EFFECTS OF FRUCTOSE.

Reaction condition: pH 5.0

Amount of substrate: 60 umol

The values in the table indicate the amounts of liberated phosphoric acid.

Keys: a, additive; b, $SnCl_2$ (0.2 mM concentration); c, $SnCl_2$ + fructose (conc., 0.25 M); d, substrate; e, choline-phosphoric acid; f, β-glycerophosphoric acid; g, phenylphosphoric acid.

VII. EFFECTS OF SUGARS ON THE INHIBITORY EFFECTS OF $SnCl_2$ ON THE

HYDROLYSIS OF SAMPLE NO. 2.

The effects of a few sugars other than fructose on the inhibitory action of $SnCl_2$ in the hydrolysis of choline-phosphoric acid were investigated, and the results are shown in Table 7.

Among various sugars, fructose showed the strongest positive effects on the hydrolysis, followed in rank by glucosamine, without appreciable difference in degree between the two. Dextrose exerted no influence on sample No. 1, but as fructose, it suppressed the inhibitory effect of $SnCl_2$ on sample No. 2. Glucosamine and lactose gave results similar to those in the hydrolysis of sample No. 1.

TABLE 7. EFFECTS OF A FEW SUGARS ON THE INHIBITORY EFFECT OF $SnCl_2$ ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID.

Reaction condition: pH 5.0.

Keys: a, additive; b, amount of liberated phosphoric acid; c, degree of acceleration; d, comparative effect of sugar; e, concentration; f, fructose; g, dextrose; h, glucosamine; i, lactose

VIII. COMPARISON OF SUBSTANCES WITH INHIBITORY EFFECTS ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID, AND EFFECTS OF FRUCTOSE.

As is well known, NaF and L-(+)-tartaric acid inhibit the activity of acid phosphatase.

TABLE 8. INHIBITORY EFFECTS OF A FEW SUBSTANCES ON THE HYDROLYSIS OF CHOLINE-PHOSPHORIC ACID AND THE EFFECTS OF FRUCTOSE

Reaction condition: pH 5.0

The values in the table indicate the amounts of liberated phosphoric acid (umol).

Keys: a, experiment No.; b, concentration of inhibitory substance; c, inhibitory substance; d, fructose; e, fructose; f, tartaric acid; g, fructose

Their inhibitory activities were compared with those of divalent stannous ion.

As shown in Table 8, the effect of divalent stannous ion is considerable. At a final concentration of 0.2 mM, the strength of such action was higher in the order of NaF - L-(+)-tartaric acid - divalent stannous ion, but no appreciable difference was noted among them at 0.02mM. Furthermore, fructose could suppress the inhibitory action of divalent stannous ion only, and its effect on NaF could not be confirmed. The inhibitory action of tartaric acid could not be controlled by fructose at all.

IX. EFFECTS OF $SnCl_2$ ON HYDROLYTIC ENZYMES OTHER THAN SEMINAL ACID PHOSPHATASE

In order to determine whether or not the actions of $SnCl_2$ and fructose on enzyme activity are confined to seminal acid phosphatase, similar experiments were performed with preputial β-glucuronidase and erythrocyte acid phosphatase whose optimum pH levels were within close range.

TABLE 9. EFFECTS OF $SnCl_2$ ON OTHER HYDROLYTIC ENZYMES

Reaction condition:	pH 5.0
Amount of substrate:	phenolphthalene-glucuronide, 1000 mmol
	phenylphosphoric acid, 60 umol
Reaction time:	1 hour

The values in the table indicate the amount of liberated phenol phthalene or phosphoric acid.

Keys: a, preputial β-glucuronidase; b, erythrocyte acid phosphatase; c, $SnCl_2$ conc.; d, additive; e, without fructose; f, 0.25 M fructose

As shown in Table 9, the former was influenced by neither divalent stannous ion nor fructose. The hydrolytic action of the latter was reduced by stannous ion to the degree the hydrolysis by seminal acid phosphatase was influenced, but no inhibitory effect of fructose on the enzyme was noted.

DISCUSSION

Choline-phosphoric acid is considered to be the most appropriate natural substrate of seminal acid phosphatase [7,8]. The author investigated the enzymic hydrolysis of choline-phosphoric acid using seminal acid phosphatase. When the choline-phosphoric acid synthesized by the author was used as the substrate, the optimum pH of seminal acid phosphatase was in the vicinity of 5.6, with a slight difference from the pH 6.3 reported by Lundquist [8]. Contrary to Hudson's observation [9], however, no choline-phosphatase with optimum pH of over 8.0 could be obtained. Comparing the speed of hydrolysis of choline-phosphoric acid with that of other substrates, the use of dilute seminal fluid as enzyme solution increased the speed slightly over the enzyme solution of purified seminal fluid. This observation led to an assumption that the semen contained a factor which contributed to the acceleration of hydrolysis. The citric acid contained in seminal fluid reportedly activates the hydrolysis of choline-phosphoric acid [8], but in the present experiment, fructose also exhibited similar effects, particularly at a final concentration of 0.25 M. Some investigators [20-28] assumed that phosphoric acid is transferred between choline-phosphoric acid and fructose, but the experiment revealed no evidence that would confirm such phenomenon.

During the present investigation, the author discovered two types of choline-phosphoric acid which exhibited different reactions toward fructose. It is believed that a number of analytical procedures employed in this experiment has almost completely clarified the differences between the two. The analysis of their emission spectra revealed that they contained numerous metallic impurities, although the quantitative determination was beyond the ability of such procedure. Together with the

results of other analyses, these metallic constituents were believed to be the cause of the difference in the behavior of fructose in enzymic hydrolysis. Among them, most responsible was tin which was found in sample No. 1 but not in sample No. 2. In fact, strong inhibitory effects of divalent stannous ion on seminal acid phosphatase was confirmed, and the degree of its effects was comparable to that of sodium fluoride or L-(+)-tartaric acid which has been known to possess similar action. There are a number of literatures discussing the inhibitory action of metallic ions on enzyme activity [24-28], but none includes divalent stannous ion as one of them. The author conducted experiments specifically to examine the effect of stannous ion on seminal acid phosphatase, and the effect of fructose on its inhibitory action. As a result, it was revealed that the action of divalent stannous ion was suppressed by fructose to a notable degree. Thus, the acceleration of the hydrolysis of sample No. 1 by fructose was interpreted as the result of fructose suppressing the inhibitory effect of divalent stannous ion on the hydrolysis. Tetravalent stannous ion failed to show such an effect. Regarding the fact that fructose completely suppresses the inhibitory action of divalent stannous ion whereas it partially controls the similar action of sodium fluoride or L-(+)-tartaric acid, such an assumption may be permitted that the different behaviors of fructose are derived from the difference in the mechanism of action toward these compounds. Experimental results also demonstrate that the suppressive action of fructose on the inhibitory effect of divalent stannous ion is exhibited only with seminal acid phosphatase. For instance, with seminal acid phosphatase, the effect of fructose shows no change when other substrate is used, whereas with erythrocyte acid phosphatase, divalent stannous ion inhibits the reaction, but its action cannot be suppressed by fructose. The differentiation

of the isoenzymes of human seminal acid phosphatase and erythrocyte acid phosphatase has been discussed in a literature [24]. The results of this investigation may be providing a new approach to such an attempt.

What must be taken into consideration is the possibility of the inhibitory action of fructose on divalent stannous ion being merely due to the degeneration of a protein. Calculating the protein levels of the enzyme solutions based on the enzyme protein level and the degree of dilution with 0.02% egg-white albumin, erythrocyte acid phosphatase showed the highest protein level (0.46 mg/ml), followed in rank by seminal acid phosphatase (0.24 mg/ml), and β-glucuronidase showed the lowest level (0.21 mg/ml). If the inhibitory effect on divalent stannous ion is derived from the general degeneration of proteins, such an effect should be stronger with smaller amount of protein. On the contrary, stannous ion showed no appreciable effect on β-glucuronidase whereas it exerted inhibitory effects on the acid phosphatases to an equal degree. Thus, the effect of stannous ion is assumed to be specific to each enzyme.

According to the pH-activity curves of two choline-phosphoric acid samples, the optimum pH of the hydrolysis of choline phosphoric acid by seminal acid phosphatase is approximately 5.6. Compared with the curve of sample No. 2 with divalent tin, the curve of No. 2 with fructose and divalent tin shows a shift of the optimum pH to the acidic side, in the vicinity of 5.0. This indicates that the action of fructose is influenced by the pH value. The affinity between acid phosphatase, the substrate, and the inhibitory substance is said to be controlled by the pH value [29], and the Km value also varies according to this factor [30]. Thus, the above-described experimental results can be related to the fact that fructose exerted some sort of influence on the action of the inhibitory substance at pH 5.0.

The difference in the velocity of hydrolysis of two choline phosphoric acid samples observed when maleic acid buffer solution was present, and the difference in the degree of suppressive action of dextrose on stannous ion may be related to the cooperative action with other metallic impurities, but the exact cause is undetermined at the present.

CONCLUSION

1. The chemical difference between two choline-phosphoric acid samples which exhibited different behaviors in their hydrolysis by seminal acid phosphatase were investigated, and the cause of the difference was pursued.

2. The emission spectra of the two samples revealed that sample No. 1 contained a trace of tin.

3. With sample No. 2, divalent tin exhibited strong inhibitory effects on seminal acid phosphatase, but such effects could be suppressed completely by fructose. Thus, thedifference in the speed of hydrolysis between the two samples was associated with the presence of divalent tin in sample No. 1.

4. The hydrolysis of No. 1 could be accelerated by sugars other than fructose, e.g., glucosamine, D-ribose, D-galactose, lactose, mannose, but not by dextrose. On the other hand, in the hydrolysis of sample No. 2, even dextrose suppressed the inhibitory action of stannoous ion

5. The inhibitory action of NaF or L-(+)-tartaric acid on seminal acid phosphatase were still noted even with β-glycerophosphoric acid or phenyl-phosphoric acid as the substrate, but tetravalent tin exhibited no inhibitory effect.

6. The inhibitory action of NaF or L-(+)-tartaric acid on seminal acid phosphatase could not be controlled by fructose.

7. The activity of human erythrocyte acid phosphatase was also

inhibited by divalent stannous ion, and the inhibitory effect could not be controlled by fructose. The activity of preputial β-glucuronidase of white rat showed no appreciable change in the presence of divalent stannous ion or fructose.

8. The pH-activity curves of the two samples indicate that the optimum pH for the hydrolysis of choline phosphoric acid by seminal acid phosphatase is 5.6 - 5.75.

The author is sincerely grateful to Prof. Mabuchi and Dr. Shirai, assistant professor, for their advice and guidance. Grateful acknowledgement is due to the Faculty of Obstetrics and Gynecology and the Faculty of Urology for the supply of enzyme samples.

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ヒト精液酸性ホスファターゼに関する研究（第1報）
精液ホスホモノエステラーゼに対する錫イオンの影響

竹内 諒吉*

精液酸性ホスファターゼ [EC 3.1.3., phosphoric monoester hydrolase, semen] に関する研究は，1935年 Kutscher ら[1]によって尾中に酸性ホスファターゼが発見された事に端を発し，本酵素がヒト精液中に多量に存在し，かつ前立腺に由来することが判明した．それらの成果はすでに Walker ら[2]，あるいはまた Schmidt[3] の諸説に紹介されている．

酵素の純化も London ら[4]，Bowman ら[5]や，最近では Ostrowski ら[6]の方法などが紹介されている．

しかしながら，酵素化学的には本酵素の基質特異性はいまだ明確さを欠き，従来の多くの研究例はいずれも天然の基質とは考えられない，グリセロリン酸，フェニルリン酸，ヒエトロフェニルリン酸などのごとき人工合成標品が用いられ，遺憾ながらこれらの成績は本酵素の本来の性質を示しているとは考え難い．Lundquist[7,8] は精液中には精漿より分泌されるコリンリン酸が存在し，これが精液酸性ホスファターゼの天然の基質であると主張しているにもかかわらず，コリンリン酸を基質としての実験は極めて少なく，Lundquist[7,8]，Hudson ら[9]，Stickland ら[10]の二，三の報告があるにすぎない．Lundquist ならびに Hudson らの研究においてもコリンリン酸水解の最適pHは一致を見ておらず，さらに水解態度も詳細に検討する必要のある事が考えられる上に，精液中にかなり含まれる果糖のごときリン酸の受容体となり得る物質がコリンリン酸を基質とした場合，ホスファターゼ活性に及ぼす影響も検討されるべきものと考えられる．本研究はこれらの問題の解明を目的として出発したものであるが，本実験中に果糖によるコリンリン酸水解に対する水解促進効果が見出された．この果糖の促進効果はコリンリン酸の合成標品によって著しい差異があり，その相異点を明確にするべく，果糖添加によって水解促進効果の発現する標品および発現しない標品の二者に関し化学的差異の追求を行なった結果，コリンリン酸標品中に不純物として含まれる錫金属の有無が両標品の果糖の差異に対する態度の相異を生ぜしめている事が疑われた．金属イオン酵素反応に一定の影響を及ぼす事は酵素学的立場から無理に難くない．ここで両標品を酵素学的あるいは化学的に比較検討を加えつつ，精液酸性ホスファターゼに及ぼす錫の影響を探索せんがために本研究を企てた結果一定の成績を得たのでここに報告する．

実験方法

I) 酵素液の調整

1) ヒト精液採取法ならびに酵素純化法

不妊症における精液検査のため神戸医科大学付属病院を訪れた男子の精液の内正常域内のものを0°C以下にて凍結保存し使用に供した．

純化方法は凍結精液をとかし，精液1容に対し0.2M酢酸緩衝液（pH 5.5）4容を加え操作の後，0°C に1時間放置し，次いで冷凍遠沈機で遠沈し（0°C，10,000 rpm，10分），精子およびタンパク質の一部を除去する．上澄を流水に対し48時間，さらに蒸溜水に対し24時間透析を行ない再び遠沈し（0°C，10,000 rpm，10分），その上澄を酵素液として使用した．以上の純化法でタンパク質に対する比活性を約10倍上昇させる事が出来た（酵素タンパク質量 1.5 mg/ml）．

2) ヒト前立腺の採取法ならびに酵素純化法

神戸医科大学付属病院において良性前立腺肥大症の患者より摘出された前立腺を直ちに凍結保存した．酵素の純化は Davidson ら[11]の方法で行なった（酵素タンパク質量 0.6 mg/ml）．

3) 赤血球酸性ホスファターゼ [EC 3.1.3.] の純化法

血液銀行より供与されたヒト赤血球浮遊液より Tuboi ら[12]の方法に準じて純化した（酵素タンパク質量 1.5 mg/ml）．

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Studies on the acid phosphatases of human semen (I): Effects of stannous ion on seminal phosphomonoesterase

By Jokichi Takenouchi (Department of Biochemistry, Faculty of Medicine, University of Kobe, Kobe)

6) シロネズミ脳膜 β-グルクロニダーゼ [EC 3.2.1] の精製法

シロネズミの脳膜に 0.25 M シュ糖液を10% (w/v) の割合に加えホモジェナイズし，0°C，100,000 g，1時間遠心，その上澄を硫安にて65%飽和とし，氷冷で17,000 g，30分遠心して生じた沈渣を生理食塩水に懸濁，4°Cで同じ生理食塩水に対し12時間透析を行ない酵素液とした（酵素タンパク質量 0.45 mg/ml）．

各酵素液は使用に際し0.02%非白アルブミンにて希釈し，V) 項に記載の条件下で基質量 60 nmol 中 15 nmol 以下の水解が得られる濃度とした．ただし，β-グルクロニダーゼにおいては基質総量 1000 nmol 中 50 nmol 以下の水解を得るように希釈した．このための各酵素液の希釈倍数は酸性ホスファターゼならびにβ-グルクロニダーゼはともに30倍，耐立酸性ホスファターゼは25倍，赤血球酸性ホスファターゼは5倍，精液は500倍が適当であった．またこれらから見て各種ホスファターゼ類のタンパク量はβ-グルクロニダーゼのタンパク量に較べ明らかに多い．

III) 基質の調製

コリンリン酸は Plimmer ら[7]の方法でカルシウム塩の形で合成し，カルシウムはシュウ酸ナトリウムで定量的に除去した．実験時に記載するごとく，コリンリン酸製品中夾雑により水解が促進される製品を No. 1，促進されない製品を No. 2 と記称する．

フェニルリン酸は Jacobson ら[8]の方法で合成した．

エタノールアミンリン酸の合成は Outhouse[9] の方法で行ない，バリウムを硫酸ナトリウムで定量的に除いた．

β-グリセロリン酸は Eastman & Kodack 社製の市販品を使用した．

以上四つの基質塩の調製に当っては，反応溶液の pH 域が4.0～5.0では基質溶液の pH を 5.0 に，また pH 域 5.5～5.9では同じく pH 5.6 に，pH 域5.8～6.8では同じく pH 6.4 に，pH 7.0～9.0 では pH 7.6 にそれぞれ補正した後基質濃度をすべて 60 μmol/ml に調製した．

β-フェノールフタレイングルクロニドの分離方法および基質溶液の調製は，Talalay ら[10] の方法に従った．本基質溶液の濃度は 1000 nmol/ml とした．

III) 酵素およびその他の添加物

染色は Merk 社製，その他の試薬は和光純薬特級または一級を使用した．これらの試薬は使用前すべて比活性度を測定し，かつペーパークロマトグラフィーにて純品であることを確認した．また第1および第2塩化鉄，フッ化ナトリウム，L(+)-酒石酸などはすべて和光純薬製のものを使用した．

IV) 緩衝液

pH 域4.0～5.9では 0.2 M 酢酸緩衝液，pH 5.8～6.8では0.1 M マレイン酸緩衝液，pH 7.0～9.1では 0.05 M ベロナール緩衝液を採用した．リン酸の転移反応を防ぐため水酸基を含む緩衝液の使用を避けた．

V) 反応条件および活性測定法

1) ホスファターゼ活性の測定

緩衝液 2.0 ml，塩加行もしくは水 1.0 ml，酵素液 1.0 ml を加え 37°C，5分のプレインキュベーションの後，基質溶液（60 μmol/ml）を 1.0 ml 加えて 37°Cで15分間インキュベートする．トリクロル酢酸 1.0 ml を添加して反応を停止せしめた後沪過し，沪液 1.0 ml について遊離リン酸量を，またその 3.0 ml について遊離コリン量を定量した．ただし，赤血球酸性ホスファターゼの活性測定の場合は，基質をβ-グリセロリン酸とし，反応時間は1時間とした．

2) リン酸の定量

Fiske-Subbarow 法[11] によった．この方法において，二糖類は終濃度 2.0 mM 以上で発色の阻害が認められるが，0.2 mM 以下では何らの影響を認めない．また四糖類は 2.0 mM においても発色を阻害しなかった．遊離コリンもなんら上記の発色を阻害しなかった．

3) コリンの定量

Entemann ら[12]の方法に準じた．

4) β-グルクロニダーゼ活性の測定

Talalay ら[10] の方法に準じた．

5) タンパク質量の定量

Lowry ら[13]の方法により測定した．

酵素活性を示す数字は全て μmol/cup または mμmol/cup で表示した．

実験結果

1) 精液酸性ホスファターゼによるコリンリン酸の水解速度

コリンリン酸（標品 No. 1）は精液酸性ホスファターゼによってかなりよく水解される．従来より基質として良く用いられて来たフェニルリン酸，β-グリセロリン酸の水解と比較して第1表にあげた．酵素液に希釈精液，

unitへの換算は表示数字をインキュベーション時間（多くは15分）で割る．

純化精液，純化前立腺抽出液などを使用したいずれの場合も，水解速度はフェエルリン酸>β-グリセロリン酸>コリンリン酸の順序でコリンリン酸に特有な酵素があるとは考え難い．しかし，希釈精液の例ではコリンリン酸の水解量は他の試料の場合と比較して著干高い．この事実から，精液中にはコリンリン酸の水解にのみ良い影響を与える物質が存在するのではないかと考えられた．

III) コリンリン酸水解に対する果糖の影響

上述のコリンリン酸水解促進因子として精液中に存在する果糖に着目し，これがコリンリン酸に及ぼす影響を検討した結果，果糖がコリンリン酸（試料 No. 1）の水解を著明に促進することを認めた（第2表）．この果糖の水解促進効果は果糖終濃度 0.05 M 以上で発現し，特に 0.25 M 以上でしかも第1図にも見られるごとく pH 5.0 で著明であった．この成績より果糖添加の実験は果糖終濃度 0.25 M，pH 5.0 の条件下で行なった．また果糖の水解促進効果は酵素濃度の増加に伴って減少する傾向が見られる．

しかしながらこの果糖の効果は第2図以後に合成したコリンリン酸試料 No. 2 を基質にした実験では反対にやや阻害を受ける傾向があり試料 No. 1 の場合と著しい差異を示した．

第1表　各種基質の水解速度の比較

酵素	基質	遊離リン酸量 (nmol/cup)	水解比 (%)
希釈精液	フェエルリン酸	40.5	100.0
	β-グリセロリン酸	22.3	55.1
	コリンリン酸	10.7	26.4
純化精液	フェエルリン酸	44.3	100.0
	β-グリセロリン酸	24.3	54.6
	コリンリン酸	8.9	19.6
純化前立腺抽出液	フェエルリン酸	42.6	100.0
	β-グリセロリン酸	23.7	55.6
	コリンリン酸	8.4	19.7

反応時間 30分
反応液の pH　基質がフェエルリン酸およびコリンリン酸の場合は pH 5.0，β-グリセロリン酸の場合は pH 5.5 で実験を行なった．

第2表　コリンリン酸試料 No. 1 水解における果糖の濃度とその促進効果との関係

果糖濃度 (M)	—	0.002	0.01	0.05	0.25	1.0
遊離リン酸量 (nmol/cup)	3.36	3.48 (+3.6%)	3.36 (± 0%)	4.92 (+46.4%)	6.48 (+92.9%)	6.72 (+100%)
遊離コリン量 (nmol/cup)	3.11	3.11 (± 0%)	3.66 (+17.7%)	4.80 (+54.3%)	6.57 (+111.5%)	6.28 (+134.1%)

酵素液 純化精液
反応条件 pH 5.0
（ ）内百分率は水解促進率を表わす．

IV) 他の基質に対する果糖の影響

第3表　他の基質に対する果糖の影響

基質 \ 果糖濃度 (M)	—	0.05	0.25
	(nmol/cup)	(nmol/cup)	(nmol/cup)
フェエルリン酸	16.4	16.1	15.4
β-グリセロリン酸	16.8	15.2	14.2
モノエタノールアミンリン酸	5.4	—	4.9

酵素液 純化精液
反応条件 pH 5.0
表中の数字は遊離リン量を表わす．

コリンリン酸以外の基質を使用した場合，果糖添加によっても水解促進効果を示さず，かえって阻害が見られる（第3表）．

IV) コリンリン酸二試料の差異の検討

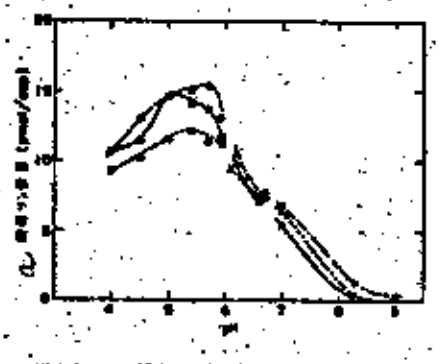
上述のごとくコリンリン酸二試料に対する果糖の効果の相異は予期せぬ事柄であり，両試料の相異点を究明すべく下記の実験を施行した．

1) コリンリン酸二試料の pH-活性曲線の比較

試料 No. 1 および No. 2 を基質に用い，果糖未添加ならびに果糖添加の条件下における pH-活性曲線を第1図および第2図に示した．

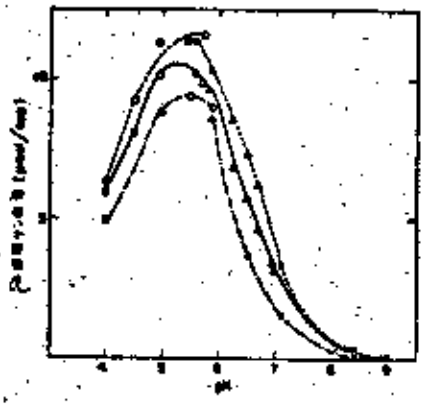
両者の共通点は果糖未添加時の場合，酵素液に粗酸精液または純化精液のいずれを用いても至適 pH は5.6で，これは Lundquist[*] が報告した pH 5.3 よりやや酸性側にある．また Hudson ら[*] が報告するごとき，pH 5.0 以上に至適 pH を有する特有なホスファターゼの存在は認められない．

第1図 コリンリン酸標品 No. 1 を基質としたときの pH 酸性曲線



反応条件 pH 5.0, 37°C, 15分
—·—·— : 150倍希釈精液果糖なし
------ : 純化精液果糖なし
——— : 純化精液＋0.25 M 果糖添加
—○— : 酢酸緩衝液
—△— : マレイン酸緩衝液
—×— : バロナール緩衝液

第2図 コリンリン酸標品 No. 2 を基質としたときの pH 酸性曲線



第1図に同じ

両標品の相異点は，第1に 0.25 M 果糖添加の場合，標品 No. 1 ではある酸性 pH 域で明らかに水解が促進されるに反し，標品 No. 2 ではそのような傾向が見られない点と，第2に標品 No. 1 の場合果糖添加で水解の至適 pH が5.6より5.0に移動する事である．第3に標品 No. 2 は標品 No. 1 に比し水解率は約3倍大きい点にあろう．これらの実験で同時に遊離コリン量も測定した結果，ほぼ等モルに遊離しており，精液酸性ホスファターゼによってコリンリン酸のリン酸部が果糖に縮移するような証拠はつかみ得なかった．

3) コリンリン酸二標品に対する他の糖類の影響

コリンリン酸標品に関して果糖以外の糖類の影響を検討した結果を第4表にあげた．標品 No. 1 では果糖の他

第4表 コリンリン酸二標品の水解に対する他糖類の促進効果の比較

糖類	比較効果	
	標品 No. 1 (%)	標品 No. 2 (%)
D-果糖	+100.0	−10.2
D-ブドウ糖	− 7.6	
D-マンノース	+ 56.6	
D-ガラクトース[*1]	+ 74.5	
D-グルコサミン	+119.7	+ 9.9
N-アセチル-D-グルコサミン	− 18.8	
D-グルクロン酸ナトリウム	−315.6	
L-アラビノース	− 83.0	
D-アラビノース	− 4.8	
D-リボース	+138.1	
D-キシロース	+ 4.8	
シュ糖	+ 8.9	
乳糖	+ 66.5	− 7.6
麦芽糖	+102.6	− 8.4
デキストリン[*2]	+ 75.6	
アデノシン[*3]	− 8.8	

標品 No. 1 を基質とした場合，果糖添加によって水解が促進された割合を +100% として他の糖類および標品 No. 2 を使用した際の促進度を比較した．

[*1] ガラクトースの水解酸より再結晶したものは，水解促進効果が著名に減少し，比較効果は +11.7 を示すのみであった．したがってこの数値に関しては検討の余地がある．

[*2] デキストリンの濃度は 0.25 M ブドウ糖と同じパーセントとした．

[*3] アデノシンの場合，濃度は 0.05 M で，0.05 M の果糖の効力と比較した．

グルコサミン，リボース，ガラクトース，乳糖，マンノースなどが水解促進効果を示すが，標品 No. 2 にあたっては他の糖類によってもやはり水解は促進されない．

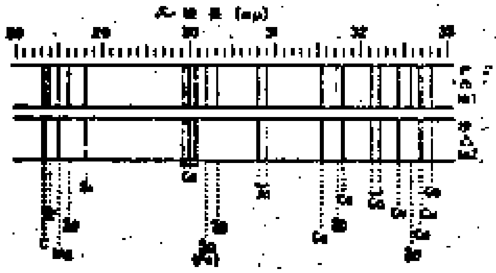
3）コリンリン酸二標品の化学的分析

両標品の酵素学的相異が標品の組成，構造などに原因する場合を考慮して化学的分析を行なった．元素分析の結果は両標品とも全く純粋のコリンリン酸カルシウムクロリドである事が確認された．また赤外線吸収スペクトルでは両標品の吸収帯は波形および吸収度ともに全く両者合致している．つぎに両標品の発光スペクトル分析の結果の一部を第3図にあげる．

スペクトル上，Ca，P の他に Cu，Al，Mg，Si，Fe などの痕跡が混在する事が証明された．とくに注意すべきは，標品 No. 1 に Sn の痕跡が認められるのに反し，標品 No. 2 ではそれが含まれていない事実である．

ここで両標品に対する果糖の効果の差異は不純物として含まれていた錫の存在の有無にあるとの推察のもとに刺激を精液酸性ホスファターゼによるコリンリン酸水解に及ぼす錫および果糖の影響を検討した．

第3図　コリンリン酸二標品の発光スペクトル



スペクトル分析の一部を転写した．

V）コリンリン酸水解における錫イオンの阻害効果と果糖の関係

1）コリンリン酸水解における $SnCl_2$ および $SnCl_4$ の影響

第5表に示すごとく，$SnCl_2$ は精液酸性ホスファターゼのコリンリン酸水解能を阻害するが，果糖を終濃度 0.25 M で添加するとその阻害が大部分除かれる．とくに 0.2 mM $SnCl_2$ による阻害が果糖添加によって取除かれる割合は標品 No. 1 を基質に用いた場合，果糖添加によって水解が促進される割合とほとんど一致する結果を得た（第2図参照）．

$SnCl_4$ では $SnCl_2$ と異なり 2.0 mM で始めて阻害効果を認めるが，その阻害が果糖の添加で除かれない点は注目すべき事である．

第5表　コリンリン酸水解における $SnCl_2$ および $SnCl_4$ の影響

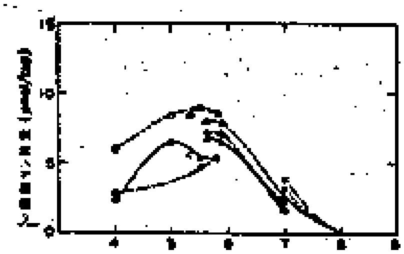
錫の濃度 ＼ 添加物		果糖なし	0.25 M 果糖
		(μmol/cup)	(μmol/cup)
$SnCl_2$	0.0 mM	10.23	—
	0.02 mM	6.17	8.45
	0.2 mM	4.25	8.58
$SnCl_4$	0.0 mM	10.90	—
	0.02 mM	10.07	10.55
	0.2 mM	8.97	8.91

反応条件 pH 5.0
表中の数字は遊離リン酸量を表わす．

2）$SnCl_2$，果糖などの存在下におけるコリンリン酸水解酵素の pH 活性曲線

コリンリン酸標品 No. 2 の水解に際し，$SnCl_2$，果糖などを添加した pH 活性曲線を第4図に示した．図を通覧するとき，添加物の有無により pH 活性曲線が著明に変動する事は明らかに認取出来る．すなわち，メディウムに何ら添加物がない時は至適 pH は 5.5，0.2 mM $SnCl_2$ 添加時は pH 5.6～5.75 の間にあり，0.2 mM $SnCl_2$ と 0.25 M 果糖の二者を添加すると至適 pH は 5.0を示す．かくのごとき至適 pH の移動は標品 No. 1 の水解に当って果糖を加えたときに見られた現象と傾向

第4図　$SnCl_2$，果糖などの存在下におけるコリンリン酸標品 No. 2 の pH 活性曲線



反応条件 pH 5.0，37°C，15分
……：添加物なし
——：0.2 mM $SnCl_2$
——：0.2 mM $SnCl_2$ + 0.25 M 果糖
—○—：酢酸緩衝液
—△—：マレイン酸緩衝液
—×—：ベロナール緩衝液

を一にする（第2図参照）．しかしながら，この実験結果において両標品間の大きな相違点は，標品 No. 2 では $SnCl_2$ 存在下で親型液を酢酸からマレイン酸にかえると反応が著しく促進されるのに反し No. 1 ではマレイン酸親型液中で反応が著しく阻害を受けた事実である．この理由については目下の処明らかでない．

VI）他種基質を用いた場合の $SnCl_2$ の阻害効果

基質のコリンリン酸を β-グリセロリン酸またはフェニルリン酸に代えた場合に $SnCl_2$ および果糖によって同様な現象が期待出来るか否かを検討した結果を第6表にあげた．

この表に示すごとく，他の基質を用いた時も上述の $SnCl_2$ および果糖の二つの効果は同様に生じ，必ずしもコリンリン酸水解に対する特有な現象とは認め難い．

第6表　他種基質を用いた場合の $SnCl_2$ の阻害効果および果糖の影響

基質 / 添加物	コリンリン酸	β-グリセロリン酸	フェニルリン酸
	(μmol/cup)	(μmol/cup)	(μmol/cup)
—	10.25	7.10	5.85
$SnCl_2$（濃度 0.2 mM）	2.89	1.85	1.74
$SnCl_2$＋果糖（濃度 0.25 M）	6.28	5.22	5.18

反応条件 pH 5.0
基質添加量 60 μmol
表中の数字は遊離リン酸量を表わす．

VII）コリンリン酸標品 No. 2 水解における $SnCl_2$ の阻害に対する二，三糖類の影響

コリンリン酸の水解を阻害する $SnCl_2$ に対して果糖以外の糖類がいかなる態度をとるかについて検討し第7表に示した結果を得た．

糖類の水解促進効果は果糖が最も強く，グルコサミンがこれに次ぐとはいえ，両者の間に認むべき差異はないと見なすべきである．コリンリン酸標品 No. 1 ではブドウ糖はその水解に著しい影響を示さなかったが，標品 No. 2 の水解に対しては果糖と同様にブドウ糖も $SnCl_2$ の阻害を排除した点が両標品の相違点である．しかし，グルコサミン，乳糖の態度は基質が標品 No. 1 のそれとほぼ一致している．

第7表　コリンリン酸水解における $SnCl_2$ の阻害効果に対する二，三糖類の影響

入添加物	遊離リン酸量	糖の促進度	糖の比較効果
	(μmol/cup)	(%)	(%)
—	10.19	—	
$SnCl_2$（濃度 0.2 mM）	3.46	—	
〃 ＋0.25 M 果糖	8.31	＋138.8	＋100.0
〃 ＋0.25 M ブドウ糖	5.42	＋ 55.7	＋ 40.1
〃 ＋0.25 M グルコサミン	8.25	＋135.9	＋ 97.9
〃 ＋0.25 M 乳糖	5.18	＋ 48.9	＋ 35.5

反応条件 pH 5.0

VIII）コリンリン酸水解における阻害物質の比較および果糖の影響

NaF および L-(+)-酒石酸が酸性ホスファターゼの阻害物質であることは周知のことである．これらの物質の

第8表　コリンリン酸に対する二，三阻害物質ならびに果糖の影響

対照番号	阻害物質 ＼ 阻害物質濃度 (mM)	0.0	0.02	0.2	2.0
		(μmol/cup)	(μmol/cup)	(μmol/cup)	(μmol/cup)
1	$SnCl_2$	10.20	6.21	4.45	—
	$SnCl_2$＋0.25 M 果糖	9.89	8.08	8.91	—
2	NaF	10.33	6.94	0.00	0.00
	NaF＋0.25 M 果糖	9.59	8.07	0.00	0.00
3	L-(+)-酒石酸	7.62	6.65	2.65	0.36
	L-(+)-酒石酸＋0.25 M 果糖	7.07	6.07	2.59	0.49

反応条件 pH 5.0
表中の数字は遊離リン酸量（μmol）を表わす．

阻害と二価陽イオンのそれとの比較を試みた。

第8表に示すごとく、二価陽イオンの阻害はかなり強い。阻害物質の特徴度0.2 mMの添加では阻害効果の強さは、NaF>L-(+)-酒石酸>二価陽イオンの関係であったが、0.02 mMの添加では三者間にほとんど差異を認めない。また果糖の阻害抑制作用は二価陽イオンに対してのみ明らかに認められ、NaFの阻害に対してはその効果は不確実であり、酒石酸の阻害にあっては全く除かれなかった。

(IX) 精液酸性ホスファターゼ以外の水解酵素に対する $SnCl_2$ の影響

上述の $SnCl_2$ ならびに果糖の酵素活性に対する態度は精液酸性ホスファターゼのみに特有なものか否かを検討するため、本酵素をその pH 至適が近似している包皮腺 β-グルクロニダーゼおよび赤血球酸性ホスファターゼについても同様の実験を試みた。

第9表　他の水解酵素に対する $SnCl_2$ の影響

	添加量 / $SnCl_2$ 濃度	果糖なし	0.25 M 果糖
		(mμmol/cup)	(mμmol/cup)
a 包皮腺β-グルクロニダーゼ	0.0 mM	48.5	47.5
	0.02 mM	45.0	42.6
	0.2 mM	45.3	47.4
		(μmol/cup)	(μmol/cup)
b 赤血球酸性ホスファターゼ	0.0 mM	9.52	6.23
	0.02 mM	6.49	5.91
	0.2 mM	3.38	3.58

反応条件 pH 5.0
基質およびその添加量　フェノールフタレイングルクロニド 1000 mμmol
フェニルリン酸 60 μmol
反応時間　1時間
表中の数字は遊離フェノールフタレインまたはリン酸量を表わす。

第9表に示すごとく、シロネズ1包皮腺 β-グルクロニダーゼは二価陽イオンではほとんど影響されないし、また果糖でも影響をうけない。ヒト赤血球酸性ホスファターゼに対して二価陽イオンは精液酸性ホスファターゼに対すると同程度の阻害作用を示したが果糖によって阻害の抑制は認められなかった。

考　　説

精液酸性ホスファターゼの天然の基質は現在の所 Lundquist[1,6] が報告したコリンリン酸が妥当なものと考えられている。筆者は精液酸性ホスファターゼを用いてコリンリン酸の酵素的水解の検討を試みた。筆者の合成したコリンリン酸を基質として用いた場合、精液酸性ホスファターゼの至適 pH は5.6付近で、従来 Lundquist[1] が報告した pH 6.3 とはわずかに異なる程度であるが、Hudson ら[1] の報告したような pH 8.0 以上に至適 pH を有する特有なコリンホスファターゼの存在は認め得なかった。コリンリン酸の水解速度を他の基質の水解速度と対比する時、酵素液に希釈精液を用いた方が純化精液を用いた時よりコリンリン酸が若干水解され易く、精液中の水解促進因子の存在が推測された。精液中に存在するクエン酸はコリンリン酸の水解を促進する事が報告されているが[1]、筆者の実験では、精液中に見出される果糖もまたコリンリン酸の水解を促進することを知った。とくに果糖終濃度 0.25 M 以上で著明であった。なおこの際、過去に研究者[2-7]が報告したごときリン酸の糖核がコリンリン酸と果糖の間で起こっているか否かを検討したが、それを裏付けるような事実は認め得なかった。

実験の進行中、果糖の効果が異なるコリンリン酸二標品が発見された。両標品の差異は各種の分析による検索の結果、ほぼ完全に解明し得たと思惟する。すなわち、発光スペクトル分析により、混入の量はスペクトル上で論じ得ないが、両標品には多数の金属が不純物として含まれている事が認められ、他の分析結果とあわせ鑑み、これらの混入金属が酵素的水解に対する果糖の態度の差を生ぜしめる原因と考えられた。とくに、標品 No. 1 に含有され、標品 No. 2 には含有されない鉛が問題となるが、事実精液酸性ホスファターゼに対する2価陽イオンの強い阻害効果が認められた。この阻害効果はすでに本酵素の阻害物質として知られるフッ化ナトリウムやL-(+)-酒石酸に匹敵する強度なものである。酵素活性に対する金属イオンの阻害作用についての報告は見られるが[2-7]、二価陽イオンの阻害効果については、未だ報告された例を見ない。ここで精液酸性ホスファターゼに対する陽イオン阻害効果とこれに対する果糖の影響を調べてみたところ、二価陽イオンの阻害効果が果糖の添加によって大部分除かれるという興味ある事実が判明した。結局、基質が標品 No. 1 の場合に生ずる果糖の促進効果は、標品 No. 1 に不純物として含まれる二価陽イオンの阻害効果が果糖により除かれた結果によって起こる現象と見なし得るであろう。なお、鉄価陽イオンでは全く阻害効果を見ない。果糖が二価陽イオンの阻害を除去

するのに反し，ファ化ナトリウムまたはL(+)酒石酸の阻害に対しては抑制作用を示す事実を考えあわせる時，この二つの阻害作用は全く別の段序の上に立つものとの推定も無理ではなかろう．また，さらに得た成績を一瞬すると，精液酸性ホスファターゼでは他の基質を用いても同様の現象が見られるのに反し，赤血球酸性ホスファターゼに対しては精液ホスファターゼにおけると同様に二価錫イオンが阻害作用を呈するが，その作用は果糖によって除かれない点から見て，二価錫イオンの阻害に対する果糖の抑制効果は精液酸性ホスファターゼに特有なものと考える．ヒト精液およびヒト赤血球酸性ホスファターゼのアイソザイムの区別に関しては報告を見る所であるが[24]，本報において得た成績は両酵素のアイソザイムを区別する一法となし得るものでなかろうか，今後の研究にゆずる．

ここで考慮すべきは，二価錫イオンに対する阻害が果して加なるタンパク質の変性に基づくものかという点である．すでに述べた酵素タンパク質および0.02%卵白アルブミン溶液による希釈倍数を勘案し，使用した酵素液のタンパク質量を算出するに，赤血球酸性ホスファターゼに最も多く（0.46mg/ml），精液酸性ホスファターゼがこれに次ぎ（0.24mg/ml），γグルクロニダーゼが最も少ない（0.21mg/ml）．もしも，二価錫の阻害効果が一般タンパク質の変性に基づくものであるならば，タンパク質量の少ないほど阻害効果が強く出るべきであるのにかかわらず，本実験ではγグルクロニダーゼに対して二価錫イオンは認むべき影響を示さず，両酸性ホスファターゼに対してほぼ同程度の阻害効果を示した．したがって，二価錫の効果は各酵素に特有な現象と推論し得る．

コリンリン酸二標品のpH活性曲線を一覧して精液酸性ホスファターゼによるコリンリン酸水解の至適pHは5.6付近と測定し得る．また二価錫を添加した標品No.2のpH活性曲線に較べ，さらにその上に果糖を添加したpH活性曲線は至適pHは酸性軽に移動しpH 5.0付近にあり，果糖の阻害抑制作用がpHにより強さの異なる事が観察された．酸性ホスファターゼと基質および阻害物質との間の飽和力はpHに関係するといわれ[25]，またK_m値もpHにより変化する[26]．上述の諸結果は果糖がpH 5.0付近にあって阻害物質の作用に何らかの影響を現わしたためと考えられる．

コリンリン酸両標品の水解速度が，マレイン酸緩衝液中で異なる点および二価錫の阻害に対するブドウ糖の抑制効果の異なる点に関しては，他の混入金属類の共同作用の影響に基づく場合も考えられるが目下のところ不明であり，今後の検討を要する問題点である．

結　言

1) 精液酸性ホスファターゼによる水解に関して，水解速度の異なる2種類のコリンリン酸標品の化学的差異を追求し，その原因を検討した．

2) 両合成標品の発光スペクトルによる精査によって，標品No.1は痕跡の錫を含むが標品No.2は錫を含まない事が証明された．

3) 標品No.2を基質に用いた場合，二価錫は精液酸性ホスファターゼに対し強い阻害効果を示し，この阻害が果糖ではほぼ完全に除かれる点から，二標品の水解速度の差異は標品No.1に2価錫が混入していたためと推定された．

4) 標品No.1は果糖以外に，グルコサミン，D-リボース，D-ガラクトース，乳糖，マンノースでも水解促進効果が認められるがブドウ糖では促進されないのに反し，標品No.2ではブドウ糖でも錫の阻害が取除かれる点で相異を示した．

5) 精液酸性ホスファターゼに対する二価錫イオンの阻害は，基質をγグリセロリン酸またはフェニルリン酸に代えても常に認められ，四価錫にはこのような阻害効果が認められなかった．

6) 精液酸性ホスファターゼに対するNaF，またはL(+)酒石酸の阻害は果糖添加によって除去されなかった．

7) ヒト赤血球酸性ホスファターゼも二価錫イオンによって阻害を受けるが，その阻害は果糖を添加しても排除されない．またシロネズミ包皮腺γグルクロニダーゼは二価錫や果糖の添加によってきしたる活性変化を認めなかった．

8) 両標品のpH活性曲線から見て，精液酸性ホスファターゼによるコリンリン酸の水解至適pHは5.6～5.75と考えられる．

稿を終るに臨んで，始終御指導，御校閲を賜わった馬淵教授ならびに白井助教授に心から深謝致します．また酵素材料を提供して頂いた神戸医大産婦人科，泌尿器科同教室に謝意を表します．

また元素分析，赤外部吸収スペクトルの測定は塩野義研究所，三宅博士，極野博士に，発光スペクトルの測定

[illegible]所生化学研究室所員，辻本氏に依頼した．ここに両氏の御厚意に深謝の微意を表します．

なお，本論文の要旨は第35回および第36回生化学会総会にて発表した．

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SPECTROSCOPIC DETERMINATION OF MANGANESE, COPPER, ALUMINUM, LEAD AND TIN IN CERTAIN VEGETABLES AND BERRIES

(Spektral'noe opredelenie marganitsa, medi, alyuminiya, svintsa i olova v nekotorykh ovoshchakh i yagodakh)

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Man needs definite amounts of various trace elements in addition to food and water, and there is hence a need for the establishment of dietary standards for these elements. However, their content in various foodstuffs is still inadequately known. Investigators working in this field are increasingly resorting to the analytical method of emission spectroscopy (Zore and Tikhonova, Stovbun et al.; Gabovich and Kul'skaya; Sokolova), because of its accuracy, rapidity, and the possibility of the simultaneous determination of several elements from the same sample.

In an earlier paper (Zore and Tikhonova) we reported on the spectroscopic determination of lead, copper and tin in fish and fish preserves. This communication describes our method for the simultaneous quantitative spectroscopic determination of manganese, copper, aluminum, lead and tin in vegetables and berries. The method was applied to the determination of the above elements in ten kinds of vegetables and berries, viz., onions, potatoes, garlic, dill, sorrel, cranberry, European black currants, "blueberry" mountain ash, [Sorbus melanocarpus], dog rose berries, and persimmon. The only available data on these four elements refer to potatoes and three of these elements in onions and dill (Stovbun et al.), no quantitative studies having been made of most of the above-mentioned elements in the other berries and vegetables. Semiquantitative spectroscopic determinations (approximate assessments) of some of the vegetables and berries we investigated have been described by Sokolova.

Our analytical method was as follows. The product was brought to a constant weight at 100—105°, then ashed in a muffle furnace at 400—450°. The ash (20 mg) was placed in the crater of a carbon electrode 3.5 mm deep and 3 mm in diameter. The sample was compacted by wetting with a drop of alcohol, dried and coated with collodion. Then the sample was ignited in an a.c. arc with a current of 10 amp and with an electrode gap of 2 mm. The spectrum was photographed with a Hilger spectrograph of medium dispersion, with a slit width of 0.005 mm, on "spectroscopic type III" photographic plates with an exposure of 60 sec.

The elements were determined by the following lines: Mn—2605.7 Å, Cu—3273.96 Å, Al—3082.2 Å, Pb—2833.07 Å and Sn—2839.9 Å. The intensity of lines was determined with reference to background with an MF-2 microphotometer. The quantitative determination of the elements investigated was performed by the method of three standards (Prokof'ev). The calibration graphs for each element were constructed by means of prepared standards with concentrations of 0.001, 0.01, 0.1 and 1 %. The standards were made with a mixture of the salts NaCl, KCl, $Ca_3(PO_4)_2$, $(NH_4)_3PO_4$, approximately corresponding to the mean concentrations of sodium, potassium, calcium, phosphorus and chlorine in the products investigated (Petrovskii).

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Concentration of Mn, Cu, Al, Pb, Sn in certain vegetables and berries, mg per 1 kg fresh weight

Foodstuff	Ash, %	Manganese			Copper			Aluminum			Lead			Tin		
		min.	max.	mean	min.	max.	mean	min.	max.	mean	min.	max.	mean	min.	max.	mean
Onions	0.65	0.64	2.73	2.10	0.30	1.03	0.80	0.32	0.76	0.50	0.05	0.07	0.06	0.07	0.11	0.09
Potatoes	0.48	0.71	2.31	2.30	0.50	0.86	0.55	1.95	2.41	2.10	0.026	0.020	0.15	0.02	0.11	0.07
Sorrel	0.88	0.34	5.05	5.50	0.80	1.86	1.28	5.10	32.00	5.26	0.08	0.29	0.10	0.02	0.07	0.06
Garlic	1.32	2.23	3.44	3.32	0.43	0.76	0.79	0.59	0.34	0.62	0.07	0.16	0.12	0.02	0.07	0.074
Dog rose	2.00	11.00	21.00	19.20	1.76	2.10	1.81	2.46	6.00	9.20	0.17	0.30	0.22	0.04	0.15	0.25
Dill	2.10	4.40	40.10	22.00	1.40	4.73	4.50	60.00	70.00	65.00	0.40	0.77	0.36	0.05	0.30	0.05
Cranberry	0.25	24.00	30.00	26.50	0.44	0.60	0.57	3.60	18.01	6.25	0.02	0.35	0.04			
Black currants	0.80	1.94	4.20	3.11	2.30	5.16	3.90	11.00	70.00	18.00	0.02	0.03	0.02	0.02	0.088	0.06
Persimmons	0.40	7.40	15.00	11.20	0.20	0.49	0.45	9.72	1.10	1.00	0.02	0.024	0.022	—	—	—
"Black-berry" mountain ash	1.03	4.25	4.84	4.19	0.51	0.66	0.62	15.5	20.8	18.9	0.18	0.51	0.50	0.02	0.05	0.03

The spectroscopic sensitivity was 0.0005% with reference to ash (0.02 mg per 1 kg fresh weight) for manganese, copper and lead, and 0.001% (0.04 mg per 1 kg fresh weight) for aluminum and tin.

The error of our method was within 6% for the determination of copper, lead and tin, and 10% for the determination of manganese and aluminum (reliability coefficient, 0.98). Concentrations of manganese, copper, aluminum, lead and tin in the products investigated, determined by means of the method elaborated, are listed in the table. Where data are not shown in the table, the concentration was too low to be measured by the method used. There was variation in the concentration of trace elements in every product, because the products were grown under a variety of conditions and on different soils. Average data are presented.

Among the products investigated, the highest manganese concentrations were found in cranberry, dill and dog rose (see table). The highest concentrations of copper occurred in black currants, dill and dog rose, and those of aluminum in dill, black currants and "black-berry" mountain ash. The results are in agreement with data previously reported by other investigators with respect to their order of magnitude (Kogan and Nasyrova, Stovbun et al.), using nonspectroscopic or spectroscopic methods.

For a fuller picture of the content of trace elements in the products investigated we made qualitative spectroscopic determinations of the following 21 elements: Mn, Pb, Sn, Si, Fe, Al, Tl, Mo, Cu, Zn, Co, Ni, Cr, Ag, Mg, V, K, Ca, Na, P and Sr. Every product contained all or nearly all these elements. All the elements investigated were detected in black currants and "black-berry" mountain ash, sorrel lacked only cobalt, garlic lacked cobalt and silver, and dog rose and dill lacked molybdenum and silver, while onions and potatoes lacked cobalt, chromium and silver.

The method can be used for simultaneous spectroscopic determination of manganese, copper, aluminum, lead and tin in vegetable products. The findings may be of help in the estimation of the content of trace elements in food.

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Can Characteristics, Metal Additives, and Chelating Agents Effect on the Color of Canned Wax Beans

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SUMMARY

The color of canned wax beans was studied in a survey of commercially packed beans in cans of different sizes and linings and in experimental packs where metals and other materials were added.

The best colored beans were seen in size 303 plain tin cans, and the worst color was found in No. 10 enameled cans. Storage led to further deterioration of the color of beans packed in enameled cans.

Addition of small amounts of iron caused considerable darkening. The darkening effect of iron was reversed by tin or stannous ions. Stannous ions were more effective than tin metal added as a foil. Aluminum and magnesium did not improve color. EDTA and citrate improved color slightly but not as much as low tin concentrations.

INTRODUCTION

When enameled cans came into use for snap beans, a feeling developed among many processors that the color of wax beans packed in enameled cans was not as bright as that of beans packed in plain cans. Such impressions were hard to substantiate when based on the products of single plants. Therefore, a cooperative study was arranged by the New York State Canners and Freezers Association and Cornell University to handle the subject.

Darkening of canned products may have many causes, but, when the type of can lining entered into consideration, it became apparent that tin and its interactions were of importance where wax beans were concerned. Hotchner *et al.* (1967) have stated that the maintenance of the desired yellow is dependent upon the solution of tin. They suggest the use of high tin fillet enameled cans in the small can sizes in order to get good color and can appearance. In connection with another vegetable, asparagus, it has long been known that iron can cause darkening (Davis *et al.*, 1961) and that tin can overcome this iron-induced darkening (Hernandez *et al.*, 1963). This study deals with the effects of different levels of iron and tin on wax bean color, as well as the effects of other metals and additives.

EXPERIMENTAL METHODS

The commercial pack was surveyed from 12 processing plants, and [illegible] ples were obtained from the plan[illegible] three different times during the p[illegible] ing season. The can variable[illegible] sizes 303 or No. 10 and plain [illegible] lytic tin or "C" enameled cans. [illegible] all, 40 separate lots of wax bean[illegible] obtained in enameled cans and [illegible] in plain cans. Of these, 56 lots [illegible] can size 303, and 47 lots were [illegible] No. 10. Beans were examined when [illegible] ceived and after 3 and 8 month[illegible] age. For the experimental pack, [illegible] ried out in the departmental [illegible] plant, heavily "gold" enameled [illegible] 303 cans (#737400, American [illegible] Co.) were used.

Color measurements of drained [illegible] beans were obtained on a [illegible] difference meter (Hunter, 1958), [illegible] 4-in. square test area, illuminat[illegible] a G.E. 4516 autolight, gave fin[illegible] ing values with a cream-colored [illegible] dard D25-303: *L*, 85.5; *a*, [illegible] *b*, 22.4. Lower *L* values indica[illegible] creasing darkness. Desirable [illegible] color characteristics of wax beans [illegible] high *L* values, low *a* values, and [illegible] *b* values which can be described [illegible] bright, pure, rich yellow color (Fr[illegible] cis, 1963).

SULTS AND DISCUSSION

The influence of can size and lining, seen in the commercial pack, will be sidered first. Then the results of use of metals and other additives be presented.

Survey of the commercial pack of wax beans. Table 1 shows a comparison of the tri-stimulus color values of canned wax beans as influenced by the use of can lining. L readings were higher for those beans packed in plain electrolytically coated tin cans. Such cans also had lower a values and higher b values. It would appear that plain tin linings resulted in a brighter canned product of a purer and more intense yellow color.

Differences due to the size of the can were also observed (Table 2). Beans from 303 cans had higher L and lower a values than those from No. 10 size cans, while there was very little difference in b values. The expected interaction between can size and can lining was also noted in that higher L and b and lower a values were found for the beans from 303 plain cans as compared to those from No. 10 enameled cans.

There was little effect of storage on the color of the beans packed in plain cans, but with enameled cans a lowering of L, a, and b values occurred when the beans were stored 8 months (Table 3). Darkening was most pronounced in No. 10 enameled cans (Table 4).

This survey led to the conclusion that plain tin cans were superior to enameled cans in packing wax beans of bright yellow color as measured on a color difference meter. It also provided the basis for further studies on the causes and prevention of darkening of wax beans in enameled cans.

Effects of treatments on color of wax beans packed in enameled cans. This work was done with Earliwax variety, and the cans used were heavily "gold" enameled size 303. The treatments were repeated five times during the 1967 season.

The darkening that had been seen previously was such that the iron was suspected as its cause. Therefore, tests were made to determine the effects of different levels of ferric iron on the color of canned material (Table 5). A lowering of the L and b values took place as the iron levels were increased. Snap beans normally contain about 10 ppm of iron (Leung *et al.*, 1952), and this amount may contribute to some darkening in the canned product. Work on asparagus has also indicated that iron is responsible for darkening with this product (Davis *et al.*, 1961), and in the case of glass-packed asparagus it is likely that the natural iron content of the spears contributes to darkening. It is evident that in the case of beans the addition of very small amounts of iron had a marked effect on color.

Such low amounts of iron probably cause discoloration through combination with tannins. The tannin content of snap beans is relatively low, most of it being concentrated in the strings and the seed coat, but there is sufficient in the epidermis to have a darkening effect. Iron combines with tannins most strongly at sites where there are orthodihydroxyl groups. The presence of iron had no effect on the amounts of carotenoids or xanthophylls in the pods.

One way to dramatically reveal the effect of iron was to remove the yellow pigments with ethanol—the pods containing high iron then had a greyish appearance with prominently colored suture lines.

Brine darkening was seen only in those cans having a high level of iron. Ferrous ions, added as ferrous sulfate, caused a darkening of wax beans and wax bean seed coats.

Adding stannous chloride to the cans had an effect opposite to that of iron. In Table 6 we see that Sn^{++} caused an increase in L, a, and b values. The

Table 1. Commercial pack of wax beans. Effect of can lining on color.[1]

	Tri-stimulus values		
	L[2]	a[2]	b[2]
Plain tin	53.8	.6	23.6
Enamel	52.4	.8	22.3

[1] Average of all can sizes and storage treatments.
[2] Averages are significantly different at 1% level.

Table 2. Commercial pack of wax beans. Effect of can size on color.[1]

	Tri-stimulus values		
	L[2]	a[2]	b
303	53.4	.0	23.0
No. 10	52.8	+1.3	22.8

[1] Average of all can linings and storage treatments.
[2] Averages are significantly different at 5%.
[3] Averages are significantly different at 1%.

Table 3. Commercial pack of wax beans. Effect of storage on color.[1]

Enameled cans	Tri-stimulus values		
	L[2]	a[2]	b[2]
Storage	52.4	.0	22.2
8 mo's storage	51.1	.5	21.5

[1] Averaged over can sizes and storage temperatures of 65 and 100°F.
[2] Averages are significantly different at 1%.
[3] Averages are significantly different at 5%.

Table 4. Extremes of color in the commercial wax bean after 8 months of storage.[1]

Size	Lining	Storage temperature °F	Tri-stimulus values		
			L	a	b
303	Plain tin	65	51.2	−.6	23.6
No. 10	Enamel	100	49.0	1.8	20.8

[1] All averages were significantly different at 1% level.

Table 5. Effect of Fe^{+++} concentrations on the color of canned wax beans.[1]

Fe^{+++} added ppm	Tri-stimulus values		
	L	a	b
0	49.0	.2 m	20.2
5	47.5 n	.3 m	19.2 n
10	46.2 n	.1 m	18.1 n
20	46.1 n	−.1 m	18.3 n
50	46.6 n	.3 m	19.1 m
100	45.6	.4 m	18.6 n

[1] Averages followed by no common letter differ significantly at 5% level.

Table 6. Effect of Sn^{++} concentrations on the color of canned wax beans.[1]

Sn^{++} ppm	Tri-stimulus values		
	L	a	b
0	49.0	.2 m	20.2
1	50.2	.5 m	21.9 m
20	51.1 m	.2 m	22.2 m n
40	51.3 m	.1 m n	22.2 m n
80	51.8 m	.1 m n	22.8 o
160	51.7 m	.6 n	22.8 m
200	51.2 m	.6 n	22.6 m o

[1] Averages followed by no common letter differ significantly at the 5% level.

Table 7. Effect of tin metal on the color of canned wax beans.[1]

	Tri-stimulus values		
	L	a	b
Control	49.0 m	.2 m	20.2
100 mg tin foil	49.6 m	.1 m	20.7
150 mg tin foil	51.3 n	−.1 m	21.4 m
100 mg tin foil plus 20 ppm Sn^{++}	51.9 n	−.1	22.2 m

[1] Averages followed by no common letter differ significantly at the 5% level.

Table 8. Comparison of iron and tin and effect of storage on color of canned wax beans.[1]

	Months storage	Tri-stimulus values		
		L	a	b
Fe^{++}, 20 ppm	3	46.1 m	−.1 m	18.5 m
	6	47.0 m	1.1 n	18.7 m
Tin foil, 100 mg	3	49.8 n	.1 m	20.7 n
	6	51.2	1.1 n	21.7
Fe^{++}, 20 ppm and tin foil, 100 mg	3	49.2 n	−.1 m	20.5 n
	6	50.1 n	1.1 n	20.9 n

[1] Averages followed by no common letter differ significantly at the 5% level.

Table 9. Effect of magnesium and aluminum on the color of canned wax beans.[1]

	Tri-stimulus values		
	L	a	b
Control	49.0 m	.2 m	20.2
Mg, 250 mg	13.6	−.3 m	16.6
Al, 204 mg	18.6 m	−.1 m	19.7
Exhausted in steam tunnel	50.1	.2 m	21.0

[1] Averages followed by no common letter differ significantly at 5% level.

Table 10. Effect of citrate on the color of canned wax beans.[1]

Citrate ppm	Tri-stimulus value		
	L	a	b
0	49.0 m	.2 m	20.2 m
10	48.8 m	.2 m	20.1 m
200	49.5 m,n	.2 m	20.6 m
400	50.3 n	0 m	21.0 n

[1] Averages followed by no common letter differ significantly at the 5% level.

Table 11. Effect of EDTA on the color of canned wax beans.[1]

EDTA ppm	Tri-stimulus values		
	L	a	b
0	49.0 m	.2 m	20.2 m
10	48.2 m	.0 m	19.9 m
20	48.9 m	.3 m,n	20.7 m
50	50.3 n	.3 m,n	21.1 n
100	50.1 n	.0 m	21.4 n

[1] Averages followed by no common letter differ significantly at the 5% level.

yellow color was purer and stronger, and the beans were brighter in appearance. A maximum effect was obtained for *L* and *b* at 20–200 ppm Sn^{++}. Furthermore, $SnCl_2$ caused a brightening and a restoration of yellow color in pods and seed coats that have been discolored by the addition of iron or that have been packed in enameled cans. The action of the Sn^{++} may be by several mechanisms. It can help to keep iron in a reduced state by the equilibrium

$$3\,Sn^{++} + 2\,Fe^{+++} \rightleftharpoons 3\,Sn^{++++} + 2\,Fe.$$

Sn^{++} can also replace Fe^{+++} in the polyphenol complex, as has been observed in rutin Fe-Sn systems (Hernandez *et al.*, 1963).

The inclusion of tin foil in the can also maintained or produced a brightening of the product; 400 mg of foil was superior to 100 mg (Table 7). The critical factor here might be the area exposed, which could be directly proportional to the weight of foil used. The foil had a weight of 926 mg per 100 cm^2. It was of interest to see that stannous chloride could improve the color in the presence of tin metal. Dame *et al.* (1959) have also reported that Sn^{++} formed bright yellow complexes more effectively than tin metal. Tin metal caused *a* values to become more negative, while Sn^{++} caused *a* values to become more positive.

Combination of iron and tin gave the results shown in Table 8, demonstrating again that the two metals have opposite effects on pod color and that Sn can overcome the effect of Fe^{+++}.

Among the effects of storage were an increase in *L*, *a* and *b* values. This was in contrast to that found in the commercial pack. The difference may be due to the type of enamel lining of the cans. For the commercial pack a light enamel was used in which there is usually some corrosion through fractures in the enamel (Hotchkiss *et al.*, 1967) that can lead to increased iron levels in the product. The later experimental packs were in very heavily enameled cans designed for fruit products.

Other metals tested were aluminum and magnesium (Table 9). Neither of these improved the appearance of the beans, and, in fact, the magnesium caused considerable discoloration and swelling of the cans.

Agents that complex iron were of some benefit in improving the color (Tables 10 and 11). The quantities needed to get marked color improvement were quite high. Even at the EDTA level of 100 ppm, the *L* and *b* were lower than that previously seen (Table 6) with 20 ppm Sn^{++}. It would appear that there are special benefits to color that derive from the presence of tin.

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Chemical Inactivation of Streptomycin

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Introduction

The problem of inactivating streptomycin is of interest in the development of a suitable sterility test as well as in the study of the mode of action and chemical nature of this antibiotic.

A preliminary report on the effect of cysteine, 2-aminoethanol hydrochloride and thioglycollic acid on streptomycin and streptothricin has indicated that streptomycin is reversibly inactivated by cysteine and that the inactivation is not a property of the sulfhydryl group nor limited to cysteine (1). Brink et al. (2), in connection with structure studies, found that carbonyl group reagents such as hydroxylamine and semicarbazide cause complete inactivation. Donovick et al. (9), have extended these studies with the carbonyl reagents semicarbazide, thiosemicarbazide, hydroxylamine hydrochloride and hydrazine hydrate, confirming and amplifying previous observations on the reaction of these agents with streptomycin. More recently the inactivation of streptomycin by several reducing agents, some of which are included in this work, has been described by Bondi et al. (3).

Following these observations we have treated streptomycin in aqueous solutions with a large number of agents, both oxidizing and reducing, in an attempt to study inactivation in terms of such reactions.

Experimental

Solutions of streptomycin hydrochloride were prepared from a dry material having a potency of 200–250 γ/mg. and were admixed with solutions of the agents listed in Tables I and II. In each case the concentrations indicated in the tables are the final concentrations after mixture with the streptomycin solutions. After allowing to stand for 1–4 hours at room temperature, the solutions were submitted for assay by a modified agar dilution streak technique patterned after that described by Wakeman and Reilly (4). Dilutions were made in increments of 10–30%, with the percentage increments decreasing as the dilutions increased. The minimal inhibiting dilution is defined as that dilution at which greater than 50% bacteriostasis of the test organism occurred.

TABLE I

Agent	Streptomycin Hydrochloride	Minimal Inhibiting Dilution		
		E. coli	*B. subtilis*	*S. aureus*
—	1 mg./ml.	340	>800	550
Potassium permanganate 0.025 *N*	—	<5	<5	<5
Potassium permanganate 0.025 *N*	1 mg./ml.	<5	<5	<5
—	1 mg./ml.	350	900	700
Potassium periodate 0.05 *M*	—	<5	<5	<5
Potassium periodate 0.05 *M*	1 mg./ml.	<5	<5	<5
—	1 mg./ml.	370	>800	500
Nitric acid 1.0 *N*	—	90	175	125
Nitric acid 1.0 *N*	1 mg./ml.	90	250	125
—	1 mg./ml.	420	>800	>800
Hydrogen peroxide 0.3%	—	80	325	250
Hydrogen peroxide 0.3%	1 mg./ml.	65	350	325
—	1 mg./ml.	440	>800	550
Sodium chlorate 0.5%	—	15	50	25
Sodium chlorate 0.5%	1 mg./ml.	7.5	30	12.5
—	1 mg./ml.	440	>800	800
Bromine[1]	—	<5	<5	<5
Bromine[1]	1 mg./ml.	440	>800	800
—	1 mg./ml.	400	>800	650
Potassium chlorate 1.0%[2]	—	<5	<5	<5
Potassium chlorate 1.0%[2]	1 mg./ml.	400	>800	650
—	1 mg./ml.	370	>800	650
Potassium chromate 5%	—	<5	<5	<5
Potassium chromate 5%	1 mg./ml.	350	>800	550
—	1 mg./ml.	380	>1000	630
Potassium bichromate 5%[1]	—	90	90	50
Potassium bichromate 5%[1]	1 mg./ml.	375	900	500

[1] Samples shaken with 1% Br_2 in CCl_4. Excess Br_2 removed after 2 hours by shaking with cyclohexene.

[2] Conducted at pH 2.0.

TABLE II

Agent	Streptomycin Hydrochloride	Minimal Inhibiting Dilution		
		B. coli	*B. subtilis*	*S. aureus*
—	1 mg./ml.	250	>500	450
Cysteine 2.0%[1]	—	15	15	15
Cystine 2.0%	1 mg./ml.	225	>500	375
—	1 mg./ml.	275	>500	>500
Hydroxylamine 0.01%	—	<5	<5	5
Hydroxylamine 0.01%	1 mg./ml.	90	250	180
—	1 mg./ml.	250	>500	500
Sodium hypophosphite 40%	—	<5	<5	<5
Sodium hypophosphite 40%	1 mg./ml.	7.5	7.5	7.5
—	1 mg./ml.	380	>1000	600
Sodium bisulfite 10%	—	140	180	180
Sodium bisulfite 10%	1 mg./ml.	100	325	130
—	1 mg./ml.	250	>500	>500
Stannous chloride 5.0%	—	65	180	110
Stannous chloride 5.0%	1 mg./ml.	70	>500	>500
—	1 mg./ml.	250	>500	475
Sodium hydrosulfite 10%	—	50	70	50
Sodium hydrosulfite 10%	1 mg./ml.	35	>500	70
—	1 mg./ml.	240	>500	375
Sodium thiosulfate 50%	—	<5	<5	<5
Sodium thiosulfate 50%	1 mg./ml.	250	>500	450

[1] Assayed after two hours' time. See Fig. 1.

A wide range of concentrations of each agent was employed but, for sake of brevity, only the lowest concentrations found effective are listed in the Tables. Since some of the agents were, in themselves, bacteriostatic in the concentrations employed, and since the minimal bacteriostatic dilution values for the streptomycin solution varied somewhat from day to day, there are included in the tables, as control values, the bacteriostatic dilutions of the streptomycin and the reagent. Thus, the minimal inhibiting dilution values listed for any one combination and the controls thereof were obtained under identical assay conditions.

Discussion

In Table I are listed the effects of 9 oxidizing agents. Potassium permanganate and potassium periodate were very effective in de-

stroying streptomycin and had little or no bacteriostatic effect in themselves under the conditions of the test in the concentrations employed. It should be pointed out that potassium permanganate, while bactericidal, is immediately reduced by constituents of the assay agar. Nitric acid also was effective in destroying streptomycin but bacteriostatic concentrations were necessary. Hydrogen peroxide and sodium hypochlorite had intermediate effects whereas the remaining reagents had no apparent effect.

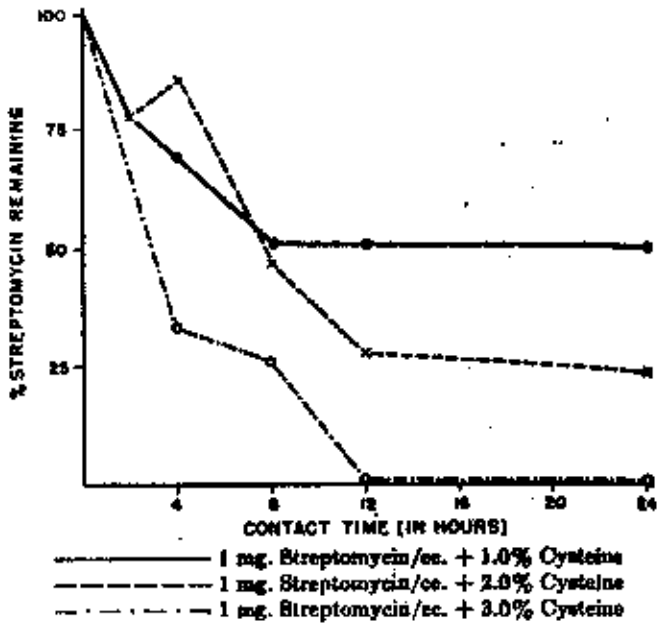

FIG. 1. Effect of Contact Time on the Inactivation of Streptomycin by Cysteine

The mechanism involved here is probably one involving oxidation of the streptomycin molecule by rather specific strong oxidants rather than the interference with an enzyme system. This is best exemplified by the effect of periodate, the action of which on polyhydroxy compounds is well known.

In Table II are listed the effects of 7 reducing agents. Included among these are cysteine and hydroxylamine which, it is recognized, may react with streptomycin through mechanisms other than redu-

tion. Of the agents tried, sodium hypophosphite in high concentrations caused essentially complete inactivation. Sodium bisulfite, cysteine, hydroxylamine, stannous chloride and sodium hydrosulfite reduced the bacteriostatic action and changed the antibacterial spectra, while sodium thiosulfate had no effect. Of considerable interest is the change in antibacterial spectra which is best exemplified by sodium hydrosulfite. The addition of this substance, while having little differential bacteriostatic effect in itself, caused a marked drop of the activity against *Escherichia coli* and *Staphylococcus aureus* but had little effect on the activity against *Bacillus subtilis*. A possible explanation for this effect may lie in the reversal of interference with specific enzyme systems. The efficacy of sodium hypophosphite may be attributed to a combination of reductive action and inhibition of streptomycin by phosphates as described by Schatz and Waksman (5) and Woodruff and Foster (6, 7).

A factor in the inactivation of streptomycin by cysteine which has not been previously described is the time effect. Fig. 1 shows that several hours are necessary for the complete reaction to take place. The results would indicate that, in this case, the inactivation must proceed through reaction with the streptomycin molecule rather than through interference with an enzyme system concerned with antibiosis. Further study of this reaction, with regard to iodine reversal (1), after prolonged contact time, would seem desirable in clarification of this postulate.

Acknowledgment

We wish to acknowledge the assistance of the Bacteriology Control Department in conductance of the agar streak plate assays employed in these studies.

Summary

1. The effects of a number of compounds, both oxidizing and reducing in nature, on the antibiotic activity of streptomycin have been described.

2. Inactivation of streptomycin may not logically be ascribed to an oxidizing nor reducing mechanism, but results from a specific reaction or an interference mechanism.

3. Potassium permanganate and potassium periodate are very specific in elimination of the antibiotic action of streptomycin. The efficacy and specificity of these agents suggests their use in development of sterility tests or chemical assay procedures for streptomycin (8).

4. Several hours are required for the inactivation of streptomycin by cysteine. This fact is suggestive of a chemical reaction between cysteine and streptomycin, rather than a reversal of the mechanism of antibiosis by cysteine.

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

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

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

INTRODUCTION

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

EXPERIMENTAL

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

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

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

Test diets

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

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

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

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

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

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

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

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

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

RESULTS

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

Up to 1953 the maximum permitted level of tin in canned food in the United Kingdom was 286 ppm. This recommendation, made in 1908, was based not on the results of biological research but on the fact that levels rarely exceeded this during routine canning of a variety of food products (Food Standards Committee, 1952). In 1953 the permitted maximum was reduced to 250 ppm (Ministry of Agriculture, Fisheries and Food Trade Press Notice No. 19537) "since a high tin content in food would be contrary to good commercial practice". Before the introduction of lacquering, the tin content frequently rose during prolonged storage to levels much in excess of the tolerated maximum (Monier-Williams, 1950) but nowadays the tin content of food in unopened cans rarely rises above 40 ppm (Adam & Horner, 1937). The tin content of food left in an opened can may rise as a result of oxidative processes (Glassmann & Barzutskaya, 1928). The levels of tin employed in these long-term tests were greatly in excess of those likely to occur in canned food. There was no evidence of chronic toxicity nor of the development of tumours in the mice as a result of the treatment.

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

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

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

Untersuchung der Wirkung Langzeitig Oral an Mäuse Verabreichten Zinks und Zinns

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

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

SIMULTANEOUS SPECTRAL DETERMINATION OF LEAD, COPPER, AND TIN IN FRESH FISH AND VARIOUS PRESERVED FOODS

by

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The determination of lead, copper, and tin in food products is of practical interest. The use of chemical methods for solving similar problems is tedious; in consequence of this, we decided to use the spectral method to which are inherent high sensitivity, rapidity, accuracy, and feasibility of determining several elements in one sample.

The methods used by us consisted of the following: 2-5mg of the product in question was dried in a dessicator at 100-105°C to obtain stable weight and was then incinerated in a muffle furnace at 400-500°C. The ash prepared in this manner, 20mg in quantity, was placed in the recess of a carbon electrode (depth 4mm, diameter 3mm) and was incinerated with an AC arc at 14A current. The spectrum was photographed with a Hilger spectrograph of medium dispersion on "spectral III" type plates with an exposure of 60 seconds. De-

termination of the element was carried out on spectral lines for lead 2833.07 Å and for tin 2839.99 Å. The intensity of the lines was determined with respect to the background on an MΦ-2 microphotometer. Quantitative determination was carried out via the three etalon method (V. K. Prokofyev, 1951).

In order to plot graduated graphs, etalons were made with concentrations of the elements in question of 0.001, 0.01, and 0.1%. Serving as the base of the etalons was a mixture of NaCl, KCl, $Ca_3(PO_4)_2$ salts, similar in sodium, potassium, calcium, magnesium, phosphorus, and chlorine content to the mineral composition of the product in question (N. Ye. Kasimova, 1961).

The sensitivity of spectral determination constitues 0.0005% with respect to the weight of ash taken for analysis (e.g., in conversion for 1 kg of fresh fish with 1.2% ash content, the amount of element discovered constituted 0.05mg).

The accuracy of the method used was established by mixing in certain concentrations of the elements in question to ash free of these elements; and subsequent

reiterated determination of these elements by the spectral method. Similar verification of accuracy via spectral methods showed that the quantities of lead, tin, and copper did not deviate by more than ±5% from their actual content in the ash.

A determination of lead, tin, and copper was carried out for fresh fish received from the Moscow commercial network. Average findings are shown in Table 1.

Table 1
Content of Lead, Tin, and Copper (in mg/kg) in Fresh Fish According to Spectral Analysis Data

Fish	Ash content,%	Lead	Tin	Copper
Flounder...	1.2	0.150	0.100	0.500-1.730
Halibut....	1.1	0.1-2.0	0.630	0.400-1.430
Cod(small).	1.0	0.200	---	0.660
Bream.....	1.2	0.125	0.150	0.270
Navaga.....	1.4	0.270	---	0.300-1.040
Ocean perch	1.0	0.400	0.170	0.460
Sprat(fresh)	1.4	---	---	0.200-0.700

The absence of an element in a sample indicates that its concentration is below the sensitivity of the method used. Quantities of lead, tin, and copper found by spectral method for fish which were studied earlier agree in magnitude with data of other authors (A. L. Adamova et al, 1949) who used other (non-spectral) methods.

Table 2 shows average findings of determinations of lead and tin in canned foods from the same batch, stored for different periods of time at 18-22°C.

Table 2

Content of Lead and Tin (in mg/kg) in Canned Foods as a function of Storage Time (average data).

Canned Food Product	Ash Content, %	Lead			Tin		
		storage time, months					
		6	12	30	6	12	30
pelamida in tomato sauce	2.4	0.58	1.31	1.72	131	230	312
scad in oil	2.2	0.20	0.31	0.76	113	152	262
sardines in oil	3.8	None	None	1.14	93	130	311
chastik in tomato sauce	2.64	0.32	0.70	2.20	190	220	377

A quantitative determination of lead and tin was also carried out for sprats. A batch of sprats (20 jars) was divided into two groups. One group was stored in a refrigerator, the other - at room temperature. Moreover, sprats were studied that had been stored in glass jars. Research took one month. Analysis data are shown in Table 3. A slight discrepancy in findings of lead and tin determination for canned foods with the data of other researchers can apparently be explained by the tremendous variations in the content of the elements in question in the fish; as well as by different storage conditions (I. Kotlyar, Y. P. Berdankina, 1941; A. P. Kizivetter, 1940).

Table 3
Content of Lead and Tin (in mg/kg) in Sprats as a function of Container and Storage Conditions (Average Data).

Container and Storage Conditions	Ash Content, %	Lead	Tin
sprats in glass jar.	11	None	6.6
sprats in tin can, kept in refrigerator	11	"	10.6
sprats in tin can, kept under domestic conditions.....	11	0.8	58
heads, bones, and sprat brine, kept under domestic conditions.....	36°	1.7	360

Thus, in consequence of our research a methodology has been developed for simultaneous spectral determination of lead, tin, and copper in food products. The sensitivity of determination of elements in ash dispersion constituted 0.0005%, accuracy - 5%. These methods enable fairly rapid (2-3 hours for a single sample and 10-15 minutes for mass analysis) determination of the content of the elements in question in food products.

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ОДНОВРЕМЕННОЕ СПЕКТРАЛЬНОЕ ОПРЕДЕЛЕНИЕ СВИНЦА, МЕДИ И ОЛОВА В СВЕЖЕЙ РЫБЕ И НЕКОТОРЫХ КОНСЕРВАХ

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Определение свинца, меди и олова в пищевых продуктах представляет практический интерес. Использование для решения подобных задач химических методов громоздко, вследствие чего мы решили применить спектральный метод, которому свойственны высокая чувствительность, быстрота, точность и возможность определять в одной пробе несколько элементов.

Применяемая нами методика заключалась в следующем: 2—5 *г* исследуемого продукта сушили в сушильном шкафу при 100—105° до получения постоянного веса и озоляли в муфельной печи при 400—450°. Приготовленную таким образом золу в количестве 20 *мг* помещали в углубление угольного электрода (глубина 4 *мм*, диаметр 3 *мм*) и сжигали в дуге переменного тока при силе тока 14 *а*. Спектр фотографировали спектрографом Хильгера средней дисперсии на пластинках «спектральные III» типа с экспозицией 60 секунд. Определение элементов производили по спектральным линиям для свинца 2833.07—А, олова 2839.99—А. Интенсивности линий определяли по отношению к фону на микрофотометре МФ-2. Количественное определение свинца, меди и олова производили методом 3 эталонов (В. К. Прокофьев, 1951).

Для построения градуировочных графиков были изготовлены эталоны с концентрациями исследуемых элементов 0,001, 0,01, 0,1%. Основой для эталонов служила смесь солей NaCl, KCl, $Ca_3(PO_4)_2$, MgCl, близкая по содержанию натрия, калия, кальция, магния, фосфора, хлора к минеральному составу исследуемого продукта (Н. Е. Кассина, 1961).

Чувствительность спектрального определения составляет 0,001% по отношению к навеске золы, взятой для анализа (например, в пересчете на 1 *кг* свежей рыбы с зольностью 1,2% количество открываемого элемента составляло 0,05 *мг*).

Точность примененной методики устанавливали путем подмешивания определенных концентраций исследуемых элементов к золам, в содержанием этих элементов, и последующим многократным определением этих элементов спектральным методом. Подобная проверка точности примененной спектральной методики показала, что отклонения определяемых количеств свинца, олова и меди от истинного содержания их в золе не превышали ±5%.

С помощью проверенной методики было проведено определение свинца, олова и меди в свежей рыбе, полученной из торговой сети Москвы. Средние результаты измерений даны в табл. 1.

Таблица 1

Содержание свинца, олова и меди (в *мг/кг*) в свежей рыбе по данным спектрального анализа

Название рыбы	Зольность (в %)	Свинец	Олово	Медь
Кильки	1,2	0,150	0,100	0,500—1,730
[illegible]	1,1	0,1—2,0	0,650	0,100—1,130
Кильки (мелкие)	1,0	0,200	—	0,660
[illegible]	1,2	0,125	0,150	0,270
[illegible]	1,1	0,270	—	0,300—1,040
Черный окунь	1,0	0,100	0,170	0,160
Белуга свежая	1,4			0,200—0,700

Отсутствие элемента в пробе указывает, что его концентрация ниже чувствительности применяемой методики. Найденные спектральным методом количества свинца, олова и меди для рыб, которые ловили раньше по порядку величин, согласуются с данными других авторов (А. Л. Адамона и др., 1949), применявших другие (неспектральные) методы.

В табл. 2 приведены средние результаты определения свинца и олова в консервах одной и той же партии, хранившихся разные сроки при t° 22°.

Таблица 2

Содержание свинца и олова (в *мг/кг*) в консервах в зависимости от времени хранения (средние данные)

Наименование консервов	Влажность (в %)	Свинец			Олово		
		срок хранения (в месяцах)					
		6	12	30	6	12	30
Сардина в томатном соусе	2,4	0,58	1,31	1,72	131	230	312
Сардина в масле	2,2	0,20	0,91	0,76	113	152	262
Шпроты » »	3,8	Нет	Нет	1,14	93	130	311
Копченая рыба в томатном соусе	2,64	0,32	0,70	2,20	190	220	377

Количественное определение свинца и олова проводили также в килках. Партия килек (20 банок) была разделена на две группы. Одна группа хранилась в холодильнике, другая — при комнатной температуре. Кроме того, были исследованы кильки, хранившиеся в стеклянных банках. Исследование велось в течение месяца. Данные анализа приведены в табл. 3. Некоторое расхождение результатов определения свинца и олова в консервах с данными других исследователей

Таблица 3

Содержание свинца и олова (в *мг/кг*) в килках в зависимости от тары и условий хранения (средние данные)

Тара и условия хранения	Зольность (в %)	Свинец	Олово
Кильки в стеклянной банке	11	Нет	6,6
Килька в жестяной банке, хранившаяся в холодильнике	11	»	10,6
Килька в жестяной банке, хранившаяся в домашних условиях	11	0,8	58
Головы, кости, рассол килек, хранившихся в домашних условиях	36*	1,7	380

по-видимому, объясняется большими вариациями содержания определяемых элементов в рыбах, а также различными условиями хранения (Н. Котляр и Е. П. Бердакина, 1941; А. Н. Кизиветтер, 1940).

Таким образом, в результате наших исследований разработана методика одновременного спектрального определения свинца, олова и меди в пищевых продуктах. Чувствительность определения элементов в навеске золы составляла 0,0005%, точность — 5%. Эта методика дает возможность достаточно быстро (2—3 часа при единичной пробе и 10—15 минут при массовом анализе) определять содержание указанных элементов в пищевых продуктах.

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