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A Modified Procedure for the Quantitative Determination of Fluoride and the Application of This Procedure to a Fluoride Balance Study in Children

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A MODIFIED PROCEDURE FOR THE QUANTITATIVE DETERMINATION OF FLUORIDE AND THE APPLICATION OF THIS PROCEDURE TO A FLUORIDE BALANCE STUDY IN CHILDREN

by

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INTRODUCTION

This research problem was done in connection with the fluoride balance studies on twelve children at Plymouth State Home. The retention balance studies of fluoride by children can be studied indirectly by measuring the amounts of fluoride excreted in the urine and feces after administration of the fluoride in water or in vitamins as sodium fluoride. The fluoride balance study was repeated, because in the previous study (19), there were no samples collected for a base day in which the fluoride was not given. The previous data also indicated that fluoride retention was merely a function of the interval of administration regardless of how the fluoride was administered.

The administration of fluoride and collection of samples was done at Plymouth State Home. Fluoride was administered to two groups of six children, each two to three years old. The children in group A were given 4.0 mg. of fluoride as sodium fluoride (8.8 mg.) in eight ounces of water and ingested by each child over a period of twelve hours or more on the first fluoride day. The children in group B were given 4.0 mg. fluoride as sodium fluoride (8.8 mg.) in vitamins and each child ingested the total amount at the beginning of the first fluoride day.

Urine samples of the children in the balance study were collected over a seventy-two hour period in twelve

hour intervals and every twenty-four hour period marked as first, second and third fluoride day. Feces were collected for a seventy-two hour period. The total amount of fluoride excreted in the feces over this period was divided by three to give the daily fecal excretion of fluoride.

Before administration of the fluoride to the children, twenty-four hour urine samples of each child were collected in twelve hour intervals for the base day of urine samples and twenty-four hour fecal samples were collected for the base day of feces of each child.

In order to quantitatively determine fluoride in urine and feces it is often necessary to evaporate or dry the samples to concentrate the fluoride. During the process the liquid sample must be alkaline to prevent the loss of fluoride compounds that are volatile in acid solution. The possible loss of small amounts of fluoride during evaporation is due to the water reacting with the samples at the drying temperature forming hydrofluoric acid, which volatilizes as HF, or reacts with any silica or silicate present, such as the glass of the container, to form volatile silicon tetrafluoride. McClure (9) noted relatively greater losses for smaller amounts of fluoride and found a difference in the magnitude of the loss of fluoride in slightly alkaline solution depending on the type of container used for the evaporation.

Biological samples for fluoride analysis are usually ashed, because of a significant quantity of organic material which is present in the samples (1) (11) (12). Some of the organic material may volatilize during the distillation to cause subsequent interference in the fluoride determination. Ashing of the biological samples must be done in the presence of a fixative, that is, a substance which will tie up all the fluoride in a non-volatile form. Examples of such fixatives are: calcium hydroxide (5), calcium oxide (12), magnesium acetate (3) and magnesium oxide.

An important factor in the isolation of fluoride is the volatility of silicon tetrafluoride and its ready formation in the presence of dehydrating acids. In 1933, Willard and Winter (18) developed a method for separating fluoride from interferences by distillation as fluoro-silicic acid and Huckabay (6) designed an apparatus heated by tetrachloroethane (b.p. 146°C) under reflux to give better and simpler control of the distillation. The advantage of steam distillation over the direct distillation is that steam distillation requires less time for the distillation of a given volume and also decreases bumping of the solution.

It was shown (7) that the best operating temperature range for the distillation of fluoride is 138-140°C.

Fluoride is quantitatively recovered in a relatively short time near that temperature. A lower temperature does not lessen the initial rate of recovery, but the last of the fluoride distills only slowly. This may be due to the presence of small amounts of complex fluorides which require the slightly higher temperature for their rapid dissociation, or it may be because of fluoride absorption on the glass surface within the still, but it is minimized by the passage of perchloric acid vapor with the steam. There is also considerable bumping as the temperature is raised and this can be reduced by the addition of steam.

The presence of boric acid, aluminum salts or gelatinous silica in the sample retards volatilization of the fluoride (13) (15). It is advisable (14) to remove chloride because the additional hydrochloric acid in the distillates may interfere in the spectrophotometric determination of fluoride. McClure (9) recommended the addition of a soluble silver salt, the sulfate or perchlorate, to the distilling chamber to avoid distilling hydrochloric acid and to fix chloride, bromide and iodide in an insoluble form.

A spectrophotometric method is the most convenient for the determination of fluoride, after it has been separated, because it enables one to determine comparatively small quantities of fluoride. Most of the colorimetric

methods for fluoride depend on the formation of a colorless complex of a metal ion with fluoride ion, upon the addition of fluoride ion to a solution of a colored metal-dye complex. Thus the decrease in color of the solution is a function of the tendency of the fluoride present to combine with the metal and this decrease in color can be used as the basis for an analytical method for fluoride.

The fluoride in the distillates were determined by a spectrophotometric method, which was developed by Megregian (10) using Zirconium-Eriochrome Cyanine R Lake. This method depends on the bleaching action of fluoride ion on the lavender colored metal dye complex formed when zirconyl nitrate, $ZrO(NO_3)_2$, reacts with the eriochrome cyanine R in acid solution. The bleaching is due to the formation of a stable colorless metal fluoride complex (16) (17).

A new reagent, Amadac-F, has recently been introduced for the determination of fluoride (2) (8). The colorimetric method is based on the blue color which fluoride ion forms with the lanthanum complexes of 3-amino-methylalizarin-N.N. - diacetic acid. In contrast to the usual bleaching of the color by fluoride, this reagent is "positive" and in addition, is free from the usual interference.

The purpose of this research was to study the above methods for fluoride separation and analysis and to improve them so that more reliable results could be obtained for the fluoride content in urine and fecal collections.

APPARATUS AND REAGENTS

Apparatus

Beckman Model DU Spectrophotometer, equipped with 1.00 cm. cells.

Huckabay distillation apparatus.

Reagents

Reagent A: Eriochrome Cyanine R (1.800 grams) was dissolved in fluoride - free water and diluted to one liter.

Reagent B: Zirconyl nitrate dihydrate (0.220 grams) was dissolved in 50 ml. water. To the zirconium solution was added 700ml. of concentrated hydrochloric acid, and the mixture was diluted to one liter with fluoride-free water.

Amadac - F: A carefully blended solid mixture of partially hydrated sodium acetate, acetic acid, stabilizers, lanthanum nitrate and 3 - amino-methylalizarin-N.N-diacetic acid (alizarin complexan). The concentrated solution was made up to contain 100mg. of Amadac-F per ml.

Sodium fluoride, 70% perchloric acid, concentrated hydrochloric acid, magnesium acetate, silver perchlorate, sodium hydroxide, sodium chloride and potassium chloride were reagent grade chemicals.

EXPERIMENTAL

1. Spectrophotometric Determination of Fluoride with Zirconium Eriochrome Cyanine R Lake.

Since there has been some disagreement as to the particular reference solution which should be used for the spectrophotometric determination of fluoride (19) using zirconium eriochrome cyanine R reagent, two kinds of reference solutions were studied and the absorption spectrum of each was determined. The Megregian reference solution (10) was prepared by adding ten ml. of reagent A to 100 ml. of fluoride-free water and to this was added ten ml. of a solution containing seven ml. of concentrated hydrochloric acid diluted to ten ml. with fluoride-free water. Another reference was a fluoride reference solution which was prepared by pipetting five ml. of each of reagents A and B into fifty ml. of a solution containing 0.050 mg. standard fluoride. These two references were used for setting the zero point of the spectrophotometer. Five ml. of reagent A and five ml. of reagent B were added to fifty ml. of water containing from 0.000 mg. to 0.050 mg. of fluoride. The absorbance of each sample was read in the wavelength range of 500 to 540 mμ against each of the two reference solutions.

The wavelengths of maximum absorption were determined. The stability of the reference solutions were also studied

by measuring the absorption at 532 mμ of the solutions against a fluoride-free water blank for a four day period.

The relationship between absorbance and concentration was studied by adding five ml. each of reagents A and B to 50.0 ml. of water containing 0.000 to 0.050 mg. fluoride. The absorbance of each of the samples was read against the two reference solutions at 532 mμ and 534 mμ. The standard curves, using both of the reference solutions were prepared by plotting the abosrbance values versus the fluoride concentration.

The interference of chloride ion in the spectrophotometric method was studied by adding from 50 mg. to 5.0 g of chloride ion as NaCl, KCl and HCl to 50.0 ml. of water containing 0.000, 0.025 and 0.050 mg. of fluoride. The reagents were added and absorbances measured against the Megregian reference solution.

2. Study of Fixing Agents

Calcium hydroxide, calcium carbonate, magnesium acetate and magnesium oxide have been recommended as fixing agents for fluoride during the evaporation and ignition process. It appeared that magnesium acetate would be a convenient reagent because of a concentrated and homogeneous solution would be prepared and thus would permit the accurate addition of reagent to each sample.

A comparison study was made of the use of magnesium acetate and calcium hydroxide as fixing agents. Known

amounts of 0.000, 0.050 and 0.100 mg. fluoride standard were added to 50.0 ml. aliquots of a urine sample, followed by either five ml. of 30% magnesium acetate or 60% magnesium acetate or twenty-five ml. of 0.5 M. calcium hydroxide as fixing agents. The samples were evaporated, ignited and distilled and the fluoride ion concentration determined. The urine sample which contained 0.000 mg. of added fluoride was used as a control blank.

Large amounts of fluoride in a large volume of urine sample were also studied. Ten ml. of 60% magnesium acetate as fixing agent was added to 200 ml. urine samples containing from 0.50mg. to 1.25mg. fluoride and also to water samples containing large amounts of fluoride. The urine and water samples were subjected to drying, ashing and distillation, followed by the spectrophotometric determination of fluoride.

3. Distillation

The determination of fluoride ion in the presence of other substances depends on the distillation from a glass vessel of fluoride as gaseous fluorosilicic acid from a solution of perchloric acid. The fluorosilicic acid is carried out of the apparatus with water vapor.

In order to determine the effect of sample volume and the rate of distillation, 0.100 mg. F^- was added in volumes of 20ml. and 5ml. to the distilling chamber, followed by 20 ml. of 70% perchloric acid. One hundred ml. of distillate was collected within fifteen minutes

for fast distillation and within thirty minutes for slow distillation.

To study the volume of distillate that must be collected for complete recovery of the fluoride, 50 ml. portions of distillates were collected in volumetric flasks from distilling chamber, which contained 20 ml. of 70% perchloric acid and 20.0 ml. of sodium fluoride solution containing 0.100 mg. of fluoride or 10.0 ml. of sodium fluoride solution containing 0.050 mg. fluoride. The rate of distillation was adjusted to approximately 3 or 4 ml. of distillate per minute. The amounts of fluoride in the distillates were determined by the spectrophotometric method.

Urine samples were also used to study the collection of distillates. Fifty ml. urine samples containing 0.050 mg. F^- and 0.100 mg. F^- as sodium fluoride, and five ml. of 60% magnesium acetate were dried and ashed. These ashed samples were distilled using 20 ml. of perchloric acid and a few drops of silver perchlorate. The distillates were collected in both 250 ml. fractions and in three fractions, consisting of two 100 ml. portions and one 50 ml. portion. The distillates were neutralized with 0.1 N sodium hydroxide to a faint pink color with the addition of two drops of phenolphthalein indicator. The fluoride was determined by the spectrophotometric method.

The chloride present in the urine samples was removed by adding silver perchlorate, which formed

insoluble silver chloride. The effect of chloride ion on the distillation of fluoride was determined by adding varying amounts of chloride ion as sodium chloride to samples containing 0.100 mg. fluoride ion. The amount of chloride added ranged from 10 mg. to 200 mg. The distillations were performed in the presence of 20 ml. of 70% $HClO_4$, with and without adding silver perchlorate to precipitate the chloride. Two hundred ml. distillates were collected and the fluoride content determined spectrophotometrically.

4. Procedure for the Determination of Fluoride in the Urine and Feces Samples Collected for the Balance Study.

The volume of each urine sample was measured and the entire sample placed in a beaker. Ten ml. of 60% aqueous magnesium acetate solution was added as a fixing agent. The mixture was evaporated down to approximately 20 - 25 ml. in a drying oven at 65°C. The low temperature was used to avoid spattering. The entire concentrated sample was transferred quantitatively to a previously ignited and weighed 30 ml. porcelain crucible. The whole sample was dried in the oven at 93°C and finally at 120°C.

Each of the feces samples was placed in a blender, followed by ten ml. of 60% aqueous magnesium acetate and enough fluoride-free water to permit thorough blending. After homogenization, the feces samples were transferred

to a preweighed beaker. The blender was rinsed with fluoride-free water and the rinsings added to the beaker. The samples were dried in an oven at about 65°C for a week, then at 93°C for three days and finally at 120°C for a day. The beakers containing the dried samples were weighed. The entire dried samples were placed in preignited and weighed crucibles.

The crucibles containing the dried urine and feces samples were placed in a muffle furnace. The temperature of the muffle furnace was raised gradually. The samples were charred for an hour at 400°C and then ignited over-night at about 600°C.

The feces samples required a temperature a little higher than 600°C and longer ignition time than the urine samples. The urine samples required an ignition temperature lower than 600°C to prevent fusion.

A portion of each ashed samples was weighed and allowed to soak in the crucible for a few minutes with fluoride-free water, then transferred into the distilling chamber through a long stemmed funnel and the crucible washed with a minimum amount of fluoride-free water and a small amount of 70% perchloric acid. Twenty ml. of 70% perchloric acid was added to the sample solution. Saturated silver perchlorate solution was added dropwise to precipitate the chloride present in the sample. The sample solution was mixed by gentle blowing through the steam inlet of the distilling vessels. The steam inlet

was stoppered and the vessel heated to the boiling point of tetrachloroethane (b.p. 146°C) within 10-15 minutes. The rate of steam passing through the solution was adjusted until approximately 4 or 5 ml. of distillate per minute were collected.

The distillates of each sample were collected in two 100 ml. and one 50 ml. volumetric flasks which contained 0.25 to 1.0 ml. of approximately 0.1N NaOH to keep it alkaline to phenolphthalein. The distillates were stored in polyethylene bottles.

Five ml. of reagent A and five ml. of reagent B were added to a 50.0 ml. portion of the distillate containing no more than 0.050 mg. of fluoride and mixed well. The spectrophotometer was set at zero absorbance at 532 mμ with the Megregian reference solution and the absorbance readings of the distillates were taken within five minutes after the reagents had been added. The amounts of fluoride were calculated from the fluoride standard curve.

Some of the urine samples became fused because of high ignition temperatures. In order to obtain a portion of the fused substance for distillation, the fused sample was dissolved in a minimum amount of 70% perchloric acid, then diluted with fluoride-free water in a 50 ml. volumetric flask and stored in polyethylene bottles. A five ml. portion of these sample solutions was distilled and fluoride determined.

5. Study of Amadac - F Reagent.

The use of Amadac - F as a reagent for fluoride was studied in order to compare its applicability with the zirconium eriochrome cyanine R reagent. Five ml. of Amadac - F concentrate were added to 25 ml. volumetric flasks which contained 0.000, 0.010, 0.025, 0.050 and 0.100 mg. of fluoride. The solutions were diluted to volume and allowed to stand for one hour. The solution which contained zero amount of fluoride was used as the blank and the absorbances of the fluoride containing samples were measured in the region 540 to 650 mμ. A standard curve was prepared by measuring the absorbance, at the wavelength of maximum absorption of solutions containing from 0.010 mg. to 0.050 mg. of fluoride against a reagent blank.

The stability of the blank was determined by measuring the absorbance of the blank against fluoride-free water. The rate of formation and stability of the fluoride complex were also studied in a pure water medium, 20% (v/v) acetonitrile-water, 20% (v/v) acetone-water and 20% (v/v) dioxane-water media (20).

This reagent was used to determine fluoride in water and urine samples. In the analysis of urine samples, 20.0 ml. of distillate was pipetted into a 25 ml. volumetric flask, followed by five ml. of Amadac - F concentrate. After an hour, the absorbance was read at 620 mμ against the blank.

RESULTS AND DISCUSSION

1. Spectrophotometric Determination of Fluoride with Zirconium Eriochrome Cyanine R Lake

The reagent, zirconium eriochrome cyanine R, is very sensitive, for even the addition of a minute amount of fluoride ion causes decolorization, that is, conversion from the dark color to a light orange color due to the formation of the colorless complex, $ZrF_6^{=}$, and liberation of the free dye.

The wavelength of maximum absorption for the Megregian reference solution was found to be 532mμ and for the fluoride reference solution, 534mμ.

Standard curves were prepared from absorbance readings at both 532mμ and 534mμ (Table I) of solutions containing the reagent and varying amounts of fluoride.

Table I.

Data for Standard Curves

Fluoride Concn.	$A_{532m\mu}$ (Megregian Ref. Soln.)	$A_{534m\mu}$ (Fluoride Ref. Soln.)
0.000mg.F/50ml.	0.796[1]	0.490[2]
0.020mg.F/50ml.	0.609	0.296
0.025mg.F/50ml.	0.565	0.243
0.050mg.F/50ml.	0.340	0.014
0.060mg.F/50ml.	0.270	
0.070mg.F/50ml.	0.202	

1. Average of ten determinations.
2. Average of three determinations.

Using the Megregian reference solution, the straight line portion is in the range of 0.000 to 0.050mg./50ml. of fluoride, with only a slight deviation in the range of 0.050 to 0.070mg.F/50ml. Lambert-Beer's Law is also followed using the fluoride reference solution, with only a slight deviation in the 0.050mg.F/50ml. sample. The results in Table II indicate that the Megregian reference solution is very stable. It was found that the addition of hydroxylamine to the Megregian reference did not increase the stability as had been previously reported (19).

Table II.

Stability of Reference Solutions

	$A^{1}_{532m\mu}$	$A^{2}_{532m\mu}$	$A^{3}_{534m\mu}$
	(distilled water used as the blank)		
1st day	0.830	0.640	1.180
2nd day	0.830	0.640	1.175
3rd day	0.828	0.645	1.170
4th day	0.830	0.642	1.160

1. Megregian reference solution.
2. Megregian reference solution + NH_2OH.
3. Fluoride reference solution.

The effect of the addition of varying amounts of chloride ion on the determination of fluoride was found (Table III) to depend on the form in which the chloride ion was added. In the case of HCl, the decrease in absorbance, which produces high results for fluoride, is probably due to the hydrogen ion attacking the dye. When

Table III.

Interference of Chloride Ion

	Absorbance*		
	(0.000mg. F/50ml.)	(0.025mg. F/50ml.)	(0.050mg. F/50ml.)
Chloride ion added as NaCl. (grams)			
0.000	0.800	0.570	0.350
0.050	0.790	-	0.340
0.100	0.790	-	0.341
0.200	0.780	-	0.335
0.500	0.770	0.548	0.330
1.0	0.740	0.540	0.348
2.0	0.740	0.550	0.375
4.0	0.760	0.620	0.480
5.0	0.770	0.655	0.525
Chloride ion added as KCl. (grams)			
0.000	0.800	0.570	0.355
0.500	0.755	0.530	0.337
1.0	0.720	0.512	0.335
2.0	0.655	0.495	0.325
4.0	0.570	0.452	0.335
5.0	0.550	0.440	0.335
Chloride ion added as HCl. (grams)			
0.05	0.775		0.335
0.10	0.760		0.329
0.20	0.720		0.321
0.50	0.660		0.315

* Absorbance measured at 532 mμ against Megregian reference solution.

small amounts of potassium chloride and sodium chloride, of the order of 100mg. Cl^-, were added to the fluoride standards, the absorbances were unaffected, but the presence of large amounts of sodium chloride resulted in high absorbance readings and low fluoride values. The effects of sodium chloride and potassium chloride are different, which might be due to the presence of large amounts of sodium or potassium ion, the sodium presumably having a different effect than the potassium. The extent of the interference depends on both the amount of the chloride salt added and the amount of fluoride present. Therefore, the reduction of the chloride ion concentration is necessary before the fluoride determination.

2. Study of Fixing Agents

The results of the comparison study of the use of the two fixing agents, calcium hydroxide and magnesium acetate are shown in Table IV. The use of magnesium acetate has increased the percentage recovery of the fluoride. The magnesium acetate solution is homogeneous rather than a suspension, which is the case of the calcium hydroxide solution (5). The use of magnesium acetate therefore, permits the addition of the same amount of fixing agent to each sample.

Table IV.

Comparison of $Ca(OH)_2$ and Mg-acetate as Fluoride Fixing Agents in Urine Samples

Fixing Agent	Sample	Fluoride Found *	%Recovery
$Ca(OH)_2$ (25ml. of 0.5M)	50ml.urine+0.050mg.F^-	0.046mg.	92.0
		0.045mg.	90.0
Mg-acetate (5ml. of 30%)	50ml.urine+0.050mg.F^-	0.049mg.	98.0
		0.050mg.	100.0
	50ml.urine+0.100mg.F^-	0.092mg.	92.0
		0.091mg.	91.0
Mg-acetate (5ml. of 60%)	50ml.urine+0.050mg.F^-	0.050mg.	100.0
		0.049mg.	98.0
	50ml.urine+0.100mg.F^-	0.093mg.	93.0
		0.096mg.	96.0

*Corrected for fluoride found in 50ml. of urine before addition of fluoride.

Table V.

Recovery of Large Amount of Fluoride Using Magnesium Acetate as Fixing Agent
(10ml. 60% Mg-acetate as fixing agent. 200ml. of distillate collected.)

Samples	Fluoride Found[1]	%Recovery
Water samples		
0.500mg.F^- in 4ml. H_2O	0.499mg.[2]	99.8
1.000mg.F^- in 8ml. H_2O	1.037mg.	103.7
1.250mg.F^- in 10ml. H_2O	1.291mg.	103.3
2.000mg.F^- in 10ml. H_2O	1.911mg.	95.6
1.000mg.F^- in 200ml. H_2O	0.914mg.	91.4
1.250mg.F^- in 200ml. H_2O	1.244mg.	99.5
Urine samples		
200ml. urine+0.500mg.F^-	0.424mg.	84.9
200ml. urine+1.000mg.F^-	0.845mg.	84.5
200ml. urine+1.250mg.F^-	0.970mg.	77.6

1. Corrected for fluoride found in the samples before addition of fluoride.
2. Calculated using weight of ashed sample.

The data in Table V shows that the recovery of fluoride is lower when a large volume of urine sample containing a large quantity of fluoride is analyzed. This, however, was not noted in the water samples. Since urine samples generally do not contain as much as 1.250mg. of fluoride, the low percentage recovery is no serious problem.

3. Recovery by Distillation

In an extensive study of the distillation of fluoride, it was found that the recovery of fluoride is inversely proportional to the still input volume (Table VI). The recovery data, as shown in Table VI, also indicates that the amount of fluoride distilled depends directly upon the rate of distillation. The samples for this study were not ashed.

Table VI.

Recovery of Fluoride by Distillation from Varying Sample Volumes and at Varying Distilling Rates.

Sample	Sample Volume	Fluoride Found[5]	%Recovery
0.100mg.F[1]	20	0.093mg.[3]	93.0
	5	0.098mg.[4]	98.0
0.050mg.F[1]	5	0.050mg.[4]	100.0
0.100mg.F[2]	20	0.069mg.[4]	69.0
0.050mg.F[2]	5	0.044mg.	88.0

1. Distilling rate: 6-7ml./min.
2. Distilling rate: 3-4ml./min.
3. Average of five determinations.
4. Average of two determinations.
5. 100ml. of distillate collected.

The percentage recovery of fluoride, when added in large amounts, as sodium fluoride, to water and urine samples and then ashed has been shown in Table IV. It appears from the data in these tables that there is good recovery of the added fluoride from either the ashed or the aqueous samples of water. The recovery of fluoride by distillation from water samples, containing from 0.050 to 2.00mg. of fluoride, was found to be satisfactory.

The recovery of fluoride from urine samples was found to be inversely proportional to the fluoride level and the volume of urine samples (Table IV and V). The results obtained also indicate that fluoride is lost during the evaporation process of the urine samples and not during the distillation.

Tables VII and VIII show the effect of the volume of distillate collected on the recovery of fluoride. These results indicate that in order to obtain nearly complete recovery of fluoride, it is necessary to collect at least 250ml. of distillate, since a significant amount of fluoride is still present in the last fractions. The volume of distillate necessary to collect for complete recovery depends on the amount of fluoride present.

It is shown from Table IX, that by collecting three fractions of distillate totaling 250ml. more fluoride is apparently recovered than when one 250ml. portion is collected. This is partly due to the fact that most of the fluoride distilled over in the first 100ml. fraction

Table VII.

Distillation Study

A. Sample: 0.100mg.F/20ml. distilled from 20ml. 70% $HClO_4$

Fraction No.	Vol. of Fraction	F in Fraction	%Recovery	Cumulative Recovery
1	50ml.	0.043mg.	43.0	43.0
2	50ml.	0.026mg.	26.0	69.0
3	50ml.	0.015mg.	15.0	84.0
4	50ml.	0.009mg.	9.0	93.0
5	50ml.	0.005mg.	5.0	98.0
6	50ml.	0.002mg.	2.0	100.0
Totals	300ml.	0.100mg.	100.0%	

B. Sample: 0.050mg.F/10ml. distilled from 20ml. 70% $HClO_4$

1	50ml.	0.025mg.	50.0	50.0
2	50ml.	0.014mg.	28.0	78.0
3	50ml.	0.007mg.	14.0	92.0
4	50ml.	0.005mg.	10.0	102.0
5	50ml.	0.000mg.	0.0	102.0
Totals	250ml.	0.051mg.	102.0%	

Table VIII.

Distillation Study in the Presence of Sodium Chloride

Sample: 0.100mg.F/20ml.+50mg. Cl/5ml. distilled from $HClO_4$

With $AgClO_4$

Fraction No.	Vol.of Fraction	F in Fraction	%Recovery
1	50ml.	0.046mg.	46.0
2	50ml.	0.030mg.	30.0
3	50ml.	0.015mg.	15.0
4	50ml.	0.007mg.	7.0
5	50ml.	0.004mg.	4.0
Totals	250ml.	0.102mg.	102.0%

Without $AgClO_4$

1	50ml.	0.050mg.	50.0
2	50ml.	0.031mg.	31.0
3	50ml.	0.014mg.	14.0
4	50ml.	0.006mg.	6.0
5	50ml.	0.004mg.	4.0
Totals	250ml.	0.105mg.	105.0%

Table IX.

Effect of Volume of Distillate on the Recovery of Fluoride

A. Single 250ml. volume of distillate collected.

Sample	Amt. of F in 50ml. distillate (found)	Aliquot factor	Total F (found)	Net F (found-blank)	Recovery
50ml.urine(blank)	0.0148mg.	5	0.074mg.		
50ml.urine+0.050mg.F^-	0.0244	5	0.122	0.048mg.	96.0%
	0.0241	5	0.121	0.047	94.0
50ml.urine+0.100mg.F^-	0.0307	5	0.154	0.080	80.0
	0.0318	5	0.159	0.085	85.0

B. 250ml. of distillate collected in three fractions.

Sample	Amt.of F in each fraction	Aliquot factor	Total F (found)	Net F (found-blank)	Recovery
50ml.urine (blank)	(1) 0.0561mg.	2			
	(2) 0.0111	2	0.072mg.		
	(3) 0.0044	1			
50ml.urine+0.050mg.F^-	(1) 0.1044	2			
	(2) 0.0135	2	0.121	0.049mg.	98.0%
	(3) 0.0030	1			
	(1) 0.0790	2			
	(2) 0.0374	2	0.121	0.049	98.0
	(3) 0.0045	1			
50ml.urine+0.100mg.F^-	(1) 0.1270	2			
	(2) 0.0340	2	0.164	0.092	92.0
	(3) 0.0032	1			
	(1) 0.1278	2			
	(2) 0.0360	2	0.168	0.096	96.0
	(3) 0.0037	1			

of distillate. The greater the amount of fluoride in the distillate, the lower the absorbancy reading because of decolorization of the dye. At lower values of absorbance the readings on the spectrophotometer become more accurate, that is, at high concentration of fluoride. So collecting 250ml. of distillate in three fractions is more reliable than collecting a single 250ml. distillate. Further studies of the distillation of three fractions versus one fraction should be done in order to clearly demonstrate and to explain a difference, if one actually exists.

In using perchloric acid in the distillation of ashed samples without the addition of silver ion, a variable quantity of chloride would come over in the distillate. From a comparison of the amounts of sodium hydroxide required to neutralize the distillate of samples to which silver perchlorate had been added and samples to which no silver perchlorate had been added, the chloride present in the sample must distill over as hydrochloric acid. The distillation of hydrochloric acid was prevented by the addition of sufficient silver perchlorate to precipitate the chloride.

The data in Table X confirms that the distillation in the presence of silver chloride precipitate was found entirely satisfactory and the silver chloride precipitate did not retain any fluoride. The results of the distillation also show that slightly high results are obtained

for fluoride when the chloride is not removed as silver chloride. The high results may be due to the fact that the hydrochloric acid which distills over requires the use of more sodium hydroxide to neutralize the distillates and it was previously shown (Table III) that the absorbance of the solutions used in the spectrophotometric method are affected by the presence of sodium chloride.

Table X.

Effect of Chloride on the Distillation of Fluoride from Water Samples

Mg.Cl^- added (NaCl)	Mg.Fluoride added	Mg.Fluoride Found*	
		With $AgClO_4$	Without $AgClO_4$
0mg.	0.100mg.	---	0.097mg.
10mg.	0.100mg.	0.102mg.	0.104mg.
20mg.	0.100mg.	0.104mg.	0.107mg.
50mg.	0.100mg.	0.102mg.	0.104mg.
100mg.	0.100mg.	0.102mg.	0.105mg.
200mg.	0.100mg.	0.099mg.	0.107mg.

*Fluoride distilled from 70% perchloric acid. 200ml. volume of distillate collected.

The recovery of fluoride was satisfactory when silver perchlorate was added to remove the chloride ion and bumping of the solution during the distillation process was decreased to some extent when the precipitate was kept continuously agitated by steam entering the mixture.

The amount of fluoride recovered varies with the type of material analyzed and the level of fluoride present. With water samples, there was almost total recovery of fluoride. However, the recovery of fluoride was considerably lower in the urine and feces samples.

4. Results of the Determination of Fluoride in Urine and Feces Samples of the Plymouth Fluoride Balance Study

The results of the fluoride analyses of the urine and feces sample of the children in the balance study are summarized in Tables XI, XII, and XIII.

In the balance study, the children in group A were given 4.0mg. of fluoride as sodium fluoride (8.8mg.) in eight ounces of water and those in group B were given 4.0mg. fluoride as sodium fluoride (8.8mg.) in vitamins. Fluoride was given in the two forms to determine if there was a significant difference in the balance of fluoride depending on the method of administration. The urine samples were collected in twelve hour periods for one day (base day) before the administration of fluoride and for three days following the administration of fluoride. The feces samples were collected in a seventy-two hour period after the administration of fluoride and a twenty-four hour period before the fluoride was given. The twelve hour urine samples were analyzed individually in order to establish a fluoride excretion pattern. The results shown in Table XII are in agreement with a previous study (19). Most of the fluoride was excreted in the

Table XI.

Fluoride Analysis of Urine and Feces Samples for Base Day

Patient	Urine Samples (mg.F) 1st 12 hrs.	2nd 12 hrs.	sum of 24 hours	Feces Samples (mg.F) 24 hours	Total Fluoride (mg.F) (urine+feces)
A-1	0.115	0.167	0.282	0.077	0.359
A-2	0.115	0.126	0.241	0.084	0.325
A-3	0.288	0.075	0.363	0.189	0.552
A-4	0.181	0.190	0.371	0.052	0.423
A-5	0.074	0.096	0.170	0.055	0.225
A-6	0.112	0.156	0.268	0.062	0.330
Average	0.148	0.135	0.283	0.087	0.370
B-1	0.163	0.162	0.325	0.062	0.387
B-2	0.131	0.122	0.253	0.041	0.294
B-3	0.163	0.183	0.346	0.105	0.451
B-4	0.150	0.096	0.246	0.122	0.368
B-5	0.080	0.093	0.173	0.041	0.214
B-6	0.147	0.150	0.297	0.125	0.422
Average	0.139	0.134	0.273	0.083	0.356

Table XII.

Fluoride Analysis of Urine and Feces Samples Collected after Administration of Fluoride

Patient	Feces (mg.F)	Urine (mg.F) (Hour at End of Collection Period) 12	24	36	48	60	72	Total (mg.F)	% Excretion
A-1	0.139	0.836	0.586	0.476	0.351	0.203	0.126	2.717	67.9
*A-2	0.224	0.256	0.446	0.412	0.400	0.252	0.376	2.366	59.2
A-3	0.479	1.046	0.159	0.714	0.408	0.354	0.232	3.392	84.8
A-4	0.354	0.693	0.719	0.390	0.225	0.229	0.272	2.882	72.1
A-5	0.865	0.794	0.589	0.270	0.092	0.218	0.258	3.086	77.2
A-6	0.785	0.795	0.531	0.642	0.175	0.191	0.153	3.272	81.8
Average	0.474	0.737	0.505	0.484	0.275	0.241	0.236	2.952	73.8
B-1	0.480	1.009	0.248	0.347	0.247	0.272	0.274	2.877	71.9
B-2	0.326	0.855	0.210	0.212	0.188	0.259	0.198	2.248	56.2
B-3	0.338	0.958	0.267	0.320	0.273	0.193	0.198	2.547	63.7
B-4	0.253	0.886	0.156	0.188	0.085	0.246	0.068	1.882	47.1
B-5	0.222	0.776	0.165	0.109	0.170	0.091	0.086	1.619	40.5
B-6	0.435	0.932	0.281	0.184	0.189	0.189	0.164	2.374	59.4
Average	0.343	0.903	0.221	0.227	0.192	0.208	0.164	2.259	56.5

*$\frac{1}{4}$oz. fluoride water loss

first fluoride day and then the excretion decreased in the second and third fluoride days.

In Table XIII is presented a summary of the total amount of fluoride excreted by each of the patients for a seventy-two hour period after the administration of the fluoride and also the amount of fluoride ingested by each patient at the start of this study. The difference between the amounts excreted and ingested is the fluoride balance. A positive balance shows retention of fluoride. For the patients in group A, the fluoride balance for the period, expressed as a percentage, ranged from +15.2 to +40.9, with an average of +26.2%. The percentage of ingested fluoride which was excreted in the urine over the same period varied from 53.6 to 72.8, with an average of 61.9. The percentage which was excreted in the feces ranged from 3.4 to 21.6, with an average of 11.9. For the patients in group B, the fluoride balance for the seventy-two hour period ranged from +28.1 to +59.5%, with an average of +43.5%. The percentage excreted in the urine varied from 34.9 to 59.9, with an average of 47.9 and that in the feces ranged from 5.6 to 12.0, with an average of 8.6. In this study, it was found that the percentage of ingested fluoride excreted in the feces was considerably lower than that excreted in the urine.

Table XIII.

Fluoride Balance Data

Patient	Fluoride Forms	Ingested Mg. of F	Mg. of Fluoride Excreted Urine	Feces	Total	Fluoride Balance (mg.)	(%)
A-1	water	4.0	2.578	0.139	2.717	+1.283	+32.1
*A-2	water	4.0	2.142	0.224	2.366	+1.634	+40.9
A-3	water	4.0	2.913	0.479	3.392	+0.608	+15.2
A-4	water	4.0	2.528	0.354	2.882	+1.118	+18.0
A-5	water	4.0	2.221	0.865	3.086	+0.914	+22.9
A-6	water	4.0	2.487	0.785	3.272	+0.728	+18.2
Average	water	4.0	2.478	0.474	2.952	+1.048	+26.2
B-1	vitamin	4.0	2.397	0.480	2.877	+1.123	+28.1
B-2	vitamin	4.0	1.922	0.326	2.248	+1.752	+43.8
B-3	vitamin	4.0	2.209	0.338	2.547	+1.453	+36.3
B-4	vitamin	4.0	1.629	0.253	1.882	+2.118	+52.8
B-5	vitamin	4.0	1.397	0.222	1.619	+2.381	+59.5
B-6	vitamin	4.0	1.939	0.435	2.374	+1.626	+40.7
Average	vitamin	4.0	1.916	0.343	2.259	+1.741	+43.5

*¼oz. fluoride water loss.

The absolute amounts of fluoride retained by the patients were not obtained because only one base day of urine and feces samples were collected (Table XI) and this is not a reliable measure of the average excretion after ingestion of natural fluoride.

On comparing group A and group B, it can be seen that there is a greater retention of the fluoride when it is given in the vitamin preparation than when it is given in water. An explanation for this is not immediately obvious. The amount of fluoride retained by the body was found to be considerably higher than expected, presumably because equilibrium was not reached in the body. It is predicted that the amount of fluoride retained by the body will decrease as equilibrium is approached (4).

Some urine samples become fused because of high ignition temperature and since these samples were hard to mix it was difficult to take a homogeneous portion for distillation. In order to determine the fluoride in these fused samples, the samples were dissolved in perchloric acid and aliquots of these solutions were used for distillation. This procedure proved to be reproducible and fairly accurate as shown by the results in Table XIV.

Duplicate portions of some ashed samples were subjected to distillation and the amount of fluoride determined in the distillate. A comparison of the

results is shown in Table XV. It appears from this data that the range of deviation from the average for the fifteen urine samples is from 0.4% to 4.0%. The mean deviation is 1.7%.

Table XIV.

Total Fluoride Found in Urine and Water Samples Using Five Ml. Aliquots of $HClO_4$ Solutions for Distillation.

Urine Sample	1st Aliquot (mg.)	2nd Aliquot (mg.)	3rd Aliquot (mg.)	Average (mg.)	Deviation (mg.)	%Devn.
1	0.253	0.253	0.255	0.254	0.001	0.4
2	0.207	0.221	0.221	0.214	0.007	3.3

Water Sample	Fluoride Found (mg.)	% Recovery
0.050mg.F^-	0.049	98.0
0.025mg.F^-	0.022	88.0

Table XV

Comparison of Total Fluoride Content as Determined on Two Different Portions of Ashed Urine Samples.

Total Fluoride in the Sample			Devn.	
1st Portion	2nd Portion	Average	from Avg.	%Devn.
0.117mg.	0.112mg.	0.1145mg.	0.0025	2.2
0.169	0.166	0.1675	0.0015	0.9
0.592	0.580	0.586	0.006	1.0
0.359	0.342	0.3505	0.0085	2.4
0.200	0.206	0.203	0.003	1.5
0.125	0.127	0.126	0.001	0.8
0.457	0.434	0.4455	0.0115	2.6
0.408	0.416	0.412	0.004	1.0
0.242	0.262	0.252	0.010	4.0
0.279	0.297	0.288	0.009	3.1
0.884	0.873	0.8785	0.0055	0.6
1.042	1.051	1.0465	0.0045	0.4
0.703	0.725	0.714	0.011	1.5
0.403	0.412	0.4075	0.0045	1.1
0.347	0.361	0.354	0.007	1.9

5. Study of Amadac-F Reagent

The wavelength of maximum absorption for the fluoride complex with Amadac-F was found to be 620mμ. This system was found to obey Beer's Law (Table XVI) from 0.000 to 0.030mg. of fluoride in twenty ml. The rate of color development and the stability of the complex formed are shown in Table XVII. It is apparent from the results in this Table, that the absorbance of the species reaches a maximum in approximately 35 to 45 minutes and the species is stable for twenty-four hours. This slow rate of color formation was overcome by the introduction of water soluble organic solvents. The rate of formation of the complex and also the reagent sensitivity were enhanced by developing the color in 20% (v/v) acetonitrile-water or acetone-water or dioxane-water media. In an acetonitrile-water medium, the absorbance of both the fluoride species and the reagent blank was found to be stabilized in 15 minutes (Table XVIII).

Table XVI.

Beer's Law Study of the Fluoride Amadac-F System

mg. F/20ml.	A* 620mμ
0.000	0.000
0.005	0.096
0.010	0.203
0.025	0.484
0.030	0.570
0.040	0.675
0.050	0.740

*Average of four determinations.

Table XVII.

Rate of Formation and Stability of the Fluoride Amadac-F Species

Time (min.)	*$A_{620m\mu}$ 0.010mg.F/20ml.	*$A_{620m\mu}$ 0.045mg.F/20ml.	Reagent Blank
5	0.108	0.670	0.540
15	0.197	0.690	0.540
35	0.210	0.715	0.540
45	0.216	0.720	---
75	0.218	0.725	---
85	0.216	0.725	---
105	0.215	0.725	---
115	0.216	0.725	0.540
24 hour	0.216	0.725	0.540
25 hour	0.206	0.715	0.540
27 hour	0.206	0.710	0.540

*Absorbance vs. distilled water

Table XVIII.

Stabilizing Effect of Organic Solvent in the Fluoride Amadac-F Species
(20% CH_3CN - H_2O)

Time (min.)	Reagent Blank	*$A_{598m\mu}$ 0.025mg.F/20ml.	*$A_{598m\mu}$ 0.050mg.F/20ml.
0	1.02	1.25	1.34
5	1.04	1.38	1.41
10	1.06	1.44	1.51
15	1.08	1.47	1.58
20	1.08	1.47	1.58
45	1.09	1.49	1.60

*Absorbance vs. distilled water

The wavelength of maximum absorption in this solvent was found to be 598mμ.

A comparison of the results obtained using the two reagents, Zr-Eriochrome Cyanine R and Amadac-F, for the determination of fluoride in water and urine sample is shown in Table XIX. The use of Zr-Eriochrome Cyanine R gives a more accurate determination of urinary fluoride than the Amadac-F reagent. The Amadac-F reagent is

Table XIX.

A Comparison of the Two Reagents:
Zr-Eriochrome Cyanine R and Amadac-F

Sample	Fluoride Found (mg.) E.C.R. Reagent	Fluoride Found (mg.) Amadac-F Reagent	% Fluoride Found E.C.R. Reagent	% Fluoride Found Amadac-F Reagent
Water Sample[1]				
0.100mg.F/5ml.	0.096[3]	0.095[3]	96.0	95.0
0.050mg.F/5ml.	0.050[4]	0.048[4]	100.0	96.0
Urine Sample[2]				
0.010mg.F/50ml.	0.009	0.008	90.0	80.0
0.025mg.F/50ml.	0.020	0.020	80.0	80.0
0.050mg.F/50ml.	0.049	0.048	98.0	96.0
0.100mg.F/50ml.	0.093	0.080	93.0	80.0

1. 200ml. distillate collected.
   Aliquote of distillate analyzed with the two reagents.
2. $Ca(OH)_2$ used as fixing agent.
   200ml. distillate collected.
3. Average of four determinations.
4. Average of two determinations.

presumably more sensitive than the Zr-Eriochrome Cyanine R to other substances distilled from the urine samples. It is possible that Amadac-F could be used for a rapid and simple method for the determination of fluoride in water samples.

SUMMARY AND CONCLUSIONS

Fluoride balance studies were carried out on two groups of six children which were two to three years old. In group A, the children ingested the fluoride in water and in group B, the children ingested fluoride as sodium fluoride in a vitamin preparation. Generally, the results indicated that the fluoride in water and vitamin was retained to some extent by the children. However, the amount retained was greater when the fluoride was given in vitamins than in water. The fluoride excretion pattern was almost identical to that of a previous study (19). Most of the fluoride was excreted in the first fluoride day and then the excretion decreased in the second and third fluoride days.

The procedure for determining fluoride in urine and feces included a preliminary ashing treatment with magnesium-acetate as a fixing agent. It also included the separation of fluoride by steam distillation as fluorosilicic acid from a perchloric acid solution, and the measurement of the decrease in absorbance of the Zr-Eriochrome Cyanine R species caused by the formation of the stable zirconium fluoride complex. Several improvements were made in various stages of this analytical procedure in order to increase the reproducibility and reliability of the results. Modifications in the procedure included the use of a different fixing agent, ignition of

the total dried sample and collection of 250ml. of distillate.

The recovery of fluoride from urine samples was found to be inversely proportional to the amount of fluoride and the volume of the sample. Over a range of 0.50 to 1.25mg. of fluoride in 200ml. of urine, the recovery was about 82% and in the range of 0.050 to 0.100mg. of fluoride in 50ml. of urine, the recovery was about 97%. Almost total recovery was obtained from water samples containing from 0.050mg. to 2.00mg. of fluoride.

The use of Amadac-F reagent was investigated. It was shown that this reagent can be used for the determination of fluoride in water. Further work on this reagent should be the development of a very rapid method, without ashing and distillation, for the determination of the fluoride content in well water.

BIBLIOGRAPHY

1. Adams, D.F., Koppe, R.K. and Mayhew, D.J., Anal. Chem., 29, 1108 (1957).

2. Belcher, R., and West, T.S., Talanta, 8, 853 (1961).

3. Crutchfield, W.E., Ind. Eng. Chem., Anal. Ed., 14, 57 (1942).

4. Ham, M.P., and Smith, M.D., J. Nutrition, 53, 215 (1954).

5. Horwitz, W. ed., Official Methods of Analysis, Assoc. Official Agr. Chem., 417 (1955).

6. Huckabay, W.B., Welch, E.T. and Mether, A.V., Anal. Chem., 19, 154 (1947).

7. Ingamells, C.O., Talanta, 9, 507 (1962).

8. Leonard, M.A. and West, T.S., J.C.S., 4477 (1960).

9. McClure, F.J., Ind. Eng. Chem., Anal. Ed., 11, 171 (1939)

10. Megregian, S., Anal. Chem., 26, 1161 (1954).

11. Miller, R.F. and Phillips, P.H., Anal. Chem., 25, 172 (1953).

12. Revinson, D. and Harley, J.H., Anal. Chem., 25, 794 (1953).

13. Rowley, R.J., Grier, J.G., and Parsons, R.L., Anal. Chem., 25, 1061 (1953).

14. Samachson, J., Slovikand, N. and Sobel, A.E., Anal. Chem., 29, 1888 (1957).

15. Smith, O.D. and Parks, T.D., Anal. Chem., 27, 998 (1955).

16. Thatcher, L.L., Anal. Chem., 29, 1709 (1957).

17. Valach, R., Talanta, 9, 341 (1962).

18. Willard, H.H. and Winter, O.B., Ind. Eng. Chem., Anal. Ed., 5, 7(1933).

19. Wright, G.R., Master's Thesis, W.M.U. Jan. 1962.

20. Yamamura, S.S., Wade, M.A. and Sikes, J.H., Anal. Chem., 34. 1308 (1962).

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