1-1962

Studies Involving Modification of the Megregian Method of Fluoride Determination and Application to Fluoride Balance Studies in Infants

Gene R. Wright

STUDIES INVOLVING MODIFICATIONS OF THE MEGREGIAN METHOD OF FLUORIDE DETERMINATION AND APPLICATION TO FLUORIDE BALANCE STUDIES IN INFANTS

A THESIS

PRESENTED TO

THE FACULTY OF THE SCHOOL OF GRADUATE STUDIES

WESTERN MICHIGAN UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE

OF

MASTER OF ARTS

IN

CHEMISTRY

BY

GENE R. WRIGHT

KALAMAZOO, MICHIGAN

JANUARY, 1962

ACKNOWLEDGEMENTS

I wish to thank my research professor, Dr. Robert Nagler, for allowing me nearly full reign over the project. I wish also to thank the various members of the Chemistry staff at Western Michigan University for their helpful suggestions.

I am indebted to The Upjohn Company and to the National Institutes of Health for supplying the financial support for the project.

Special appreciation is due to a number of people who more out of interest than for the monetary pittance afforded them helped in the experimental procedures. These include Thomas Davies (who performed the bulk of the spectrophotometric determinations), Margaret DeForest, Catherine Comstock (who did most of the large number of weighings involved), Susan Englebreit, Toni Tetzlaff, Joyce Comstock, and Ernest Clapp.

Also included should be my wife, Delores, who helped by arranging the bibliography, by being an inspiration at times when the task seemed hopeless, and by patiently putting up with the inconveniences that my full-time studentship has put on our family life.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd.)

INTRODUCTION

At the request of Dr. Frederick Margolis, M.D., and in association with The Upjohn Company, this research problem was initiated as a participation in the investigation of whether or not fluoride ingested as a constituent of the Upjohn 47-M liquid vitamin preparation is retained in an infant's body to the same extent as is fluoride ingested as the sodium salt by itself--either in solution or in tablet form. The retention patterns may be studied indirectly by measuring the amounts of fluoride excreted in the urine and in the feces during each of a series of equal time intervals after administration of the various fluoride preparations.

Because of experience in these laboratories it was decided to attempt to use the method of fluoride analysis developed by Megregian (44). His procedure basically involves the determination of fluoride spectrophotometrically by reduction of the degree of complex formation between eriochrome cyanine R and zirconium by preferential complexing of the latter with fluoride. To accomplish the objectives of this study it was necessary to exhaustively examine various aspects of the Megregian method.

In order to link this thesis project to the total clinical investigation, there is presented herein a brief history of the samples prior to their receipt for analysis in these laboratories. Also reported is our treatment of the samples prior to application of the Megregian method.

EXPERIMENTAL PROCEDURE AND DISCUSSION

Administration of Fluoride and Collection of Samples.

Fluoride was administered in each of three forms to different male infants, ages one to two years, at the Plymouth State Home and Training School, Plymouth, Michigan. Medication A (Med. A) consisted of 2.0 mg. of fluoride as sodium fluoride in eight ounces of water, and was given over a period of eight hours or more, starting at the beginning of the first collection period. Medication B (Med. B) was to consist of 2.0 mg. of fluoride incorporated into a liquid vitamin preparation, but one-half dose (1.0 mg. fluoride) was given due to a miscalculation. The entire amount was given at the beginning of the first collection period. Medication C (Med. C) consisted of 2.0 mg. of fluoride as sodium fluoride in tablet form, and again the entire dose was given at the beginning of the first collection period.

Urine and feces specimens were collected for a forty-eight hour period after the administration of the various fluoride preparations. Urine specimens were collected in eight consecutive six-hour periods. Feces specimens were pooled over longer time intervals, generally about twenty-four hours. Unfortunately, no base line samples were collected before administration of the fluoride preparations.

Med. A (fluoride water) was administered to three different patients, Med. B (fluoride-vitamin preparation) was administered to four different patients, and Med. C (fluoride tablet) was administered to three different patients. In some cases the administration of the various fluoride preparations was repeated on the same patients for a second forty-eight hour test period. One each of the Med. A and

Med. C patients and two Med. B patients were so treated. However, the data for this second test period for the Med. A and Med. C patients had to be discarded. This was due for the former to the loss of some of the medication and for the latter to imcomplete collection of samples. The second test period data for the two Med. B patients involved was complete and was averaged in with the first test period results of all four Med. B. patients.

Drying and Ashing the Samples.

In order to prevent fluoride from being lost in the drying process, the samples must be made alkaline. Moreover, a "fluoride fixative" is needed to prevent loss of fluoride in the ashing process. Calcium hydroxide (6, 11, 50, 51, 53) accomplishes both of these functions, and was used in the present series of determinations. Fixatives that have been used by other authors include aluminum nitrate (12), calcium acetate (10, 60), calcium carbonate (66), calcium phosphate (54), metallic copper (66), magnesium acetate (9, 60), a mixture of magnesium acetate and magnesium oxide (21), potassium hydroxide (31), and sodium hydroxide (23, 37, 53).

In the present series of determinations a 0.5 M lime suspension was prepared as described in Official Methods of Analysis of the Association of Official Agricultural Chemists (32), except that the suggested extra purification of the calcium oxide was not carried out. Determinations of the fluoride content of the calcium oxide (Fisher, low in fluorine) used were made in order to provide a correction, but the amount present (about .003 to .004 mg. per 25 ml. 0.5 M suspension) was deemed insignificant when compared with other sources of error in

the experimental procedure.

The fluoride-free water used above and elsewhere in the experiments was prepared by distilling from slightly alkaline solution water that had been previously distilled and passed through a Bantum Replacement Cartridge for the Barnstead deionizing apparatus.

To each urine sample in the polyethylene bottle in which it had been collected was added twenty-five ml. of freshly shaken lime suspension. The contents were then shaken and poured into a preweighed pyrex beaker. The bottle was then rinsed with fluoride-free water and the rinsings were added to the beaker.

Each of the stool samples was placed in a Waring blender, followed by twenty-five ml. of freshly shaken lime suspension and enough fluoride-free water to permit thorough blending. After homogenization the contents were poured into a preweighed pyrex beaker. The blender was then rinsed with fluoride-free water and the rinsings were added to the beaker.

The beakers were then placed in a drying oven at about 150° F (65° C) for one week. Since not all the samples were completely dry, the temperature was then raised to about 200° F (93° C) for three days. Drying was still not complete in some of the urine samples--due partly to crust formation--so the temperature was raised to about 250° F (121° C) for one day. The beakers containing the dried samples (plus calcium oxide) were weighed, and the weight of dry samples (plus calcium oxide) was calculated for each specimen. Portions of the dried samples were placed in previously ignited and weighed porcelain crucibles. The crucibles containing the sample portions were then weighed, and the weight of dried sample (plus calcium oxide) portions were calculated. The sample (plus calcium oxide) portions in the crucibles

were charred for one hour at about 400° C in a muffle furnace with the door open. In this time charring was complete enough that there was no danger of flaming with concomitant loss of sample on stronger heating. The samples were then ashed over-night in a muffle furnace at about 600° C.

It has been warned (32) that some contamination of samples by fluoride from the walls of the muffle furnace may occur. On determination of the samples it was found that the results from one portion of a particular sample indicated over fifty times as much fluoride in the total sample as did the results from another portion of the same sample. On checking back it was found that the first mentioned portion had accidentally been heated to 1000° C in the muffle furnace. Since this was the only variable in the procedure it seems probable that at this higher temperature fluoride was more effectively driven out of the walls of the muffle furnace and was trapped by reaction with the calcium oxide present in the sample portion. This suggests a way of preparing a muffle furnace so that the danger of its contributing to fluoride contamination during the ashing process may be reduced. The furnace need only be set at 1000° C while containing vessels filled with calcium oxide until analyses show that no additional fluoride is being released.

Distillation of Ashed Samples.

Fluoride is often separated from other constituents of the ashed sample by some form of distillation process (1, 6, 7, 10, 11, 14, 15, 19, 21, 22, 25-28, 35, 37, 39, 40, 42, 43, 46, 47, 49, 50, 51, 54-56, 60, 61, 63, 64). Steam distillation (1, 21, 37, 46, 51) from acid

solution--usually some modification of the Willard and Winter method (6, 15, 25, 26, 28, 40, 49, 63, 64)--is often used. In some cases a double distillation (6, 15, 26, 63) is necessary. The acids most frequently used are sulfuric (6, 15, 21, 22, 27, 35, 37, 42, 50, 54-56, 61) and perchloric (1, 10, 11, 15, 19, 21, 46, 51). Depending on the conditions of distillation, fluoride comes over as silicon tetrafluoride, SiF_4 (22, 42, 50, 55), as hydrofluosilicic acid, H_2SiF_6 (19, 35, 39), or as hydrofluoric acid, HF (21). Several authors (6, 43, 46) have suggested adding silver sulfate or silver perchlorate to the distillation vessel to prevent evolution of chloride as HCl.

In the present study steam distillation of hydrofluosilicic acid was carried out from perchloric acid using special fluorine stills (33). Each ashed sample was soaked in the crucible for about one-half an hour with fluoride-free water. The soaked ash was then broken up with a stirring rod, slurried, and poured into the still through a long-stemmed funnel. (In cases in which the ash could not be broken up with the stirring rod, an agate mortar and pestle set was used.) Then as much as possible of the sample clinging to the stirring rod and the crucible was rinsed into the still with fluoride-free water. Twenty ml. of 70% perchloric acid was to be added to the still. A portion of this was first used to rinse the crucible and stirring rod. The rinsings were added to the still. The remainder of the perchloric acid was then added to the still. Silver perchlorate solution was then added to precipitate the chloride present in the sample, thus preventing its evolution as HCl. Ten ml. of approximately .34 M silver perchlorate was sufficient for the stool samples. Since the urine samples contained more chloride than did the stool samples, larger amounts of silver perchlorate were necessary.

Either saturated or nearly saturated silver perchlorate was therefore used for the urine samples in order to keep the volume added at a minimum. The amount required varied from twelve drops to over two-hundred drops. The silver perchlorate was added to these samples in twelve drop portions with mixing after each addition. When the suspension formed on initial addition of silver perchlorate became coagulated it was known that enough had been added (48).

Initially, much trouble with bumping was encountered in the distillation. Bumping was often so vigorous that some of the solution in the still splashed over into the receiving flask. One of the critical factors seemed to be the intensity of the flame produced by the Fischer burner under the still heating-bath jacket. In order to permit accurate reproduction of a given flame intensity the positions of the needle valve and the air inlet adjustment on the Fischer burner, once set, were left alone, and the gas flow was regulated by adjusting the gas line valve until the fluid in a manometer attached to the system reached a given level. Ethanol was used as the manometer fluid since it has a relatively low density and thus provides greater sensitivity.

A satisfactory procedure was worked out as follows: 1. After addition of ashed sample, perchloric acid, silver perchlorate, and rinsing water to the still, these were mixed by gently blowing through the steam inlet. Otherwise the more dense perchloric acid tended to layer on the bottom; then when the still was heated the perchloric acid would be heated above the boiling point of water and, on mixing with the solution floating above it, would produce violent bumping. 2. With the steam inlet tube clamped shut, the Fischer burner was turned on at a gas pressure of about 8 mm. of ethanol. 3. When the volume of solution

in the still fell below forty ml., steam was admitted at such a rate that about five ml. of distillate was produced per minute; and the gas pressure was turned up to 20 mm. of ethanol.

The source of the steam used above was that described by Huckabay et al. (33). A heating coil--made by wrapping nichrome wire in a spiral around a piece of glass tubing--is immersed in water in a two-liter florence flask. This water must be made slightly alkaline with sodium hydroxide in order to prevent any fluoride present from distilling over with the steam and contaminating the samples. Adding phenolphthalein provides a convenient method for making sure the solution is basic. The coil is connected to a powerstat for easy control of the rate of steam production.

The distillates were collected in 100 ml. volumetric flasks. One ml. of approximately 0.1 M sodium hydroxide and five drops of 0.2% phenolphthalein indicator were placed in each collection flask before collection of distillate was begun. This helped determine in doubtful cases whether or not bumping over of the acid solution from the stills into the collection flasks occurred during the beginning of the distillation, the time of greatest danger of bumping. The one ml. of base added to the collecting flask was enough to neutralize about one-half of the acid that normally distilled over. Collection of distillate was stopped when only about 99 ml. had been collected. The 0.1 M sodium hydroxide was then used to titrate the remainder of the acid that had distilled over. This served to give an indication as to whether or not bumping had occurred during the latter part of the distillation and an estimation of the acid content of the normal distillates. The distillates were then made to volume in the collection

flasks, mixed by inversion, and stored in 4 ounce polyethylene bottles pending determination.

With seven out of the first twelve urine ash samples an inordinate amount of acid was found in the distillate. This was at first attributed to bumping, but addition of silver perchlorate to one of the distillates produced a copious white precipitate--indicating that chloride had been distilled over as HCl. Thus the need, as discussed above, was discovered for the addition of more silver perchlorate to the urine ashes in the stills than to the stool ashes.

When bumping over into the distillate occurred the distillation was nevertheless continued until about 100 ml. had been collected. This solution was placed in a beaker and neutralized with 1 M sodium hydroxide. One ml. of the base was added in excess. Then the solution was evaporated to dryness in the drying oven, and the residue subjected to redistillation. Stored distillates in which mold developed and distillates containing an excess amount of acid due to distillation of HCl were treated in a similar manner.

Determination of Fluoride.

Many methods of determining the isolated fluoride have been described in the literature (1-8, 10, 13-18, 20, 22, 24, 27, 29, 34, 36, 38, 39, 41-43, 45, 47, 50-52, 57-59, 61-65). Titration and colorimetric techniques were most often used. Titrants used include ferric chloride (50), sodium hydroxide (34), thorium nitrate (1, 5-7, 10, 14-18, 39, 43, 59, 64), zirconyl nitrate (7), and zirconyl chloride (36, 41). Indicators used for the various titrations include sodium alizarinsulfonate (1, 2, 43), alizarin red (16, 17), aluminum--aluminon

lake (5), zirconium--alizarin (10, 18, 39, 59, 64), and zirconium--purpurin (7, 36, 41). Colorimetric methods include the use of the following reagent pairs: alizarin and thorium nitrate (97), aluminon and aluminum (5, 6), salicylic acid and ferric ammonium sulfate (8), ammonium thiocyanate and ferric chloride (24), titanium and hydrogen peroxide (13-15, 45, 57, 62, 63), titanium and perhydrol (20), alizarin and zirconium or zirconyl ion (51, 58, 65), sodium alizarinmonosulfate and zirconyl chloride (3), and benzidine and molybdate (an indirect method in which silicon is determined) (22, 42). The basis for most of the above colorimetric procedures is the partial decolorization of complexes formed between the given reagent pairs by competition of fluoride ion for the metal ion involved. Gravimetric techniques (29, 34) have also been employed.

In the present study a modification of the colorimetric method developed by Megregian (44) is used. Megregian's method involves partial dissociation by fluoride of a complex formed by the interaction in acid solution of zirconyl nitrate, $ZrO(NO_3)_2$, with eriochrome cyanine R. The structural formula of this dye is shown in figure I in the appendix. Both the free dye and the complex are colored, but they have absorption maxima at different wavelengths.

Two reagents, called A and B, are used in the Megregian method. Reagent A consists of 1.800 grams of eriochrome cyanine R dissolved in distilled water and diluted to one liter. Reagent B is made up by dissolving 0.220 grams of zirconyl nitrate dihydrate in about 50 ml. of distilled water, adding 700 ml. of concentrated hydrochloric acid, and diluting to 1 liter with distilled water. Five ml. of each of reagents A and B is pipeted into fifty ml. of the unknown fluoride

solution. The resulting solution is well mixed, then read on a spectrophotometer against a reference solution made up by adding 5 ml. of reagent A and 5 ml. of hydrochloric acid of the same concentration as in reagent B to 50 ml. of fluoride-free water. The results are compared with a previously run standard curve.

However, in measuring the absorbances of standard fluoride samples with a Beckman DU spectrophotometer against the reference solution suggested by Megregian it was found that the absorbances of the standards relative to the reference solution were not stable, but dropped off with time (See appendix, table I.). Megregian had stated that a 3° C difference in temperature produces a change in relative absorbance equivalent to 0.01 p.p.m. of fluoride. It was therefore suspected that the changes in absorbance observed might be due to heating of the solutions in the cell compartment of the spectrophotometer. It should be noted that the spectrophotometer suggested by Megregian is the Beckman B spectrophotometer. This instrument has a more open cell compartment than does the DU, and thus might be less likely to cause heating of the cells. That such heating does take place in the DU was confirmed by use of a previously calibrated thermistor inserted through a hole bored in a special cell compartment cover into a water-containing cuvet (absorption cell) in the cell compartment. As is shown in figure II in the appendix, after an initial lag of about one minute, the water was heated at a rate of about one-half a degree centigrade per minute for about six minutes, after which the rate of further heating declined. By allowing the setup to equilibrate over a long period of time it was shown that the temperature in the cell compartment was about 35° C.

The Megregian scheme involves measuring a solution of high absolute

absorbance against another solution of high absolute absorbance. Thus the changes in relative absorbances observed could have stemmed from changes in the absolute absorbance of either the reference solution or the fluoride solution. Or the absolute absorbances of both the reference solution and the fluoride solution might have changed, but at different rates and/or to different degrees. Several efforts were therefore made to develop methods of measuring the absolute absorbances of the various solutions. In one attempt a variety of inorganic salts that form colored aqueous solutions were used in an effort to find stable reference solutions. Among these cobalt nitrate seemed most suitable. Solutions of high absorbance can be made up, and the slope of its spectral curve is slight at the wavelength (528 millimicrons) involved. Stepwise reference solutions were made up with equal concentration increments such that equal absorbance increments of about .5 were obtained. The first reference was measured against air, the second reference against the first, etc., until the absorbance range of the fluoride solutions was reached. This tedious procedure, however, did not give consistent results. A similar series of steps was attempted by using varying amounts of reagents A and B in water solution, again without good results.

Another attempt at measuring the absolute absorbances was made using the Beckman B spectrophotometer. This involved adjusting the absorbance to .000 at sensitivity setting 1 with air as the reference, reading the absorbance of a cobalt nitrate reference solution at a sensitivity setting of 3, setting the absorbance at .000 for the cobalt nitrate solution at a sensitivity of 1, and reading the absorbance of the fluoride solutions at a sensitivity setting of 2 or 3. Each increase of 1 in sensitivity setting makes the indicated absorbance about

.5 less than the actual relative absorbance. This method permitted the elimination of several of the cobalt nitrate reference solution "steps." But results were again not reproducible. A similar method was tried with the 0.1 selector switch setting on the DU, again without much success. At this setting the transmittance scale reading must be multiplied by 0.1 (divided by 10) to get the true transmittance. Therefore "1" must be added to the absorbance reading to obtain the true absorbance, since:

$$A_{0.1} = \log 1/T_{0.1}$$

$$\text{and } A_1 = \log 1/T_1 = \log 1/(0.1T_{0.1}) = \log 1/T_{0.1} + \log 1/0.1$$

$$= A_{0.1} + 1$$

$A_{0.1}$ and $T_{0.1}$ are the absorbance and transmittance readings, respectively, at selector switch setting 0.1; A_1 and T_1 are these readings at selector switch setting 1, the normal postion. Use of the 0.1 setting thus increases the measuring range of the instrument by one absorbance unit. However, the sensitivity is very poor at this setting. A relatively large movement of the absorbance scale produces only a slight movement of the null-meter needle. Thus accurate results cannot be obtained.

It may be appropriate at this point to indicate a disadvantage common to methods involving the spectrophotometric measurement of the <u>difference</u> in absorbance between two solutions each of rather high absolute abosrbance. This is that any volumetric errors involved in making up the solutions will be multiplied. For example, suppose the absolute absorbances of a range of concentrations of unknowns from 10 to 0 p.p.m. are from 3.000 to 3.500, respectively, and that a reference solution of absolute absorbance 3.000 is used. A solution containing 5 p.p.m. should give an absorbance of 3.250, providing Beer's Law holds.

If a volumetric error of 1% were made in preparing this solution the error in the absolute absorbance would be 1%, or .032. The error in concentration of unknown, however, would be .032 x 10 p.p.m./.500, or .64 p.p.m., or 13%.

It should be mentioned also that the setting of the "sensitivity" control of the DU affects the accuracy with which the spectrophotometric measurements can be made. With the sensitivity control turned fully counterclockwise the absorbance setting must be changed by .016 to produce a change of 1 division on the null-meter; whereas with the sensitivity control fully clockwise the absorbance setting must be changed by only .0016 to produce the same change on the null-meter. Thus the null adjustment is ten times as sensitive when the sensitivity control is full clockwise as when it is full counterclockwise. The fact that the null needle tends to fluctuate in the former case, thus making reading more difficult, is more than compensated for by the increase in accuracy. Despite the vascillations of the needle, absorbance readings are more reproducible.

In an early experiment a portion of reference solution was heated to about 38° C, and its transmittance dropped to about 95% (i.e., its absorbance went up) compared with that of unheated reference solution. As the temperatures equilibrated, however, the absorbances of the two samples approached each other. This suggests that the heating of the reference solution produced no permanent effect, but merely shifted the equilibrium conditions. A repetition of this experiment confirmed that the heating and cooling have no permanent effect on the absorbance of the reference solution, but that the absorbance is significantly different at different temperatures.

If the absorbances of the fluoride solutions do not change appreciably with temperature, or at least do not change as rapidly as does the absorbance of the reference solution, the previously mentioned drop in absorbance of the fluoride samples with respect to the reference solution (See table I in the appendix.) is explained. This view gains support from the fact that while there was considerable change in the absorbances of the fluoride solutions relative to the reference solution, there was comparatively little change in the absorbances of the fluoride solutions relative to each other. Contrast tables I and II in the appendix. The differences in the values listed in table II for each concentration are within the experimental error in setting and reading the spectrophotometer, especially when it is considered that each value in table II was calculated from two absorbance readings, each with its own inherent error.

Unfortunately, although this data was gathered at the outset of the project, the above relationship derived from the data was not noticed at the time. It was not until much later (but before the unknown fluoride samples were analyzed) that use of the Megregian reference solution was abandoned in favor of a suitable fluoride solution as a reference. This was done previous to the above discovery in an attempt to reduce the number of variables involved in the procedure.

Another departure from the Megregian procedure was the addition of hydroxylamine hydrochloride to both reagents A and B. It had been noticed that the reagent B had developed a distinct yellow tinge. The same results were obtained on making up a fresh solution of the reagent using different sources of zirconyl nitrate and hydrochloric acid. Previous reports in the literature (6, 39) have

pointed out that the presence of oxidizing agents such as free chlorine tends to cause bleaching of dyes being used in colorimetric methods. It has been suggested that sodium nitrite (39) or hydroxylamine hydrochloride (6) be used to reduce any strong oxidizing agents present, thus preventing the bleaching of the dye. That there indeed was an oxidizing agent present in reagent B was confirmed by shaking a portion of this reagent with potassium iodide solution in the presence of carbon tetrachloride. On settling, the carbon tetrachloride layer was colored deep violet, indicating that the iodide had been oxidized to free iodine. Since reagent B contains some nitrate (from the zirconyl nitrate) and hydrochloric acid in fairly concentrated form, it is suspected that these two react to produce free chlorine.

Twenty-five drops of 1 M hydroxylamine hydrochloride per fifty ml. of reagent B were sufficient to cause the yellow color to almost (if not completely) disappear and to remain at a very low level. This amounts to about 0.025 moles of hydroxylamine hydrochloride per liter of reagent. To be on the safe side reagents A and B were both made 0.04 M with respect to hydroxylamine hydrochloride. Except for this they were made up according to the directions cited above given by Megregian.

Addition of sodium nitrite to the reagent B produced a deepening of the yellow color; and the formation of a colorless gas which turned brown and acquired a pungent odor on exposure to air indicated the production of nitric oxide, NO. Sodium nitrite was therefore deemed unsatisfactory for addition to reagent B.

Megregian (44) suggested the use of sodium arsenite to prevent bleaching by oxidizing agents, but this suggestion had been over-

looked at the time. On testing the arsenite compound later it was found that it produced fizzing when added to reagent B, and did not permanently destroy the yellow coloration. So it also was considered unsatisfactory for addition to reagent B. Megregian had suggested its use as an additive to the developed fluoride solutions or to reagent A. There is no reason to believe that it might not have been satisfactory when employed in this way. But since the hydroxylamine hydrochloride seemed to work, its use was continued.

Besides its intended function as an antioxidant the hydroxylamine hydrochloride appears to bestow other desirable qualities on reagent A and on the developed fluoride solutions. The reagent A containing hydroxylamine hydrochloride does not foam as much on mixing as does the ordinary reagent A. Neither are bubbles as likely to form in the pipet used to measure out the reagent, and if bubbles do form they are much easier to break. Previously it had been necessary to completely empty the pipet on the frequent occasions when bubbles formed. Also, developed fluoride solutions can be more easily poured from the polyethylene bottles into the cuvets without spilling. These facts suggest that an increase in surface tension is involved. Another advantage of using reagents containing hydroxylamine hydrochloride is that cleaning of the cuvets after use is made easier. It had been found previously that on rinsing and filling the cuvets with distilled water following a determination the water slowly took on a pink coloration. This indicated that some of the eriochrome cyanine R had been adsorbed to the walls of the cuvets. It was found that soaking in dilute nitric acid was necessary to remove the adsorbed dye. When reagents containing hydroxylamine hydrochloride are used, the dye

does not cling as tenaciously and may be removed by repeated rinsing with water. Here too, however, the use of dilute nitric acid facilitates removal of the dye.

In the light of factors discussed above the following procedure was evolved for the determination of fluoride samples. Fifty ml. each of three fluoride standards (.050 mg./50 ml., .025 mg./50 ml., and .000 mg./50 ml.) and a number (usually about fifteen) of unknowns were pipetted into 4-ounce polyethylene bottles. Five ml. of the reagent A made 0.04 M in hydroxylamine hydrochloride was added to each bottle, followed by five ml. of the reagent B made 0.04 M in hydroxylamine hydrochloride. The contents of each bottle were mixed thoroughly. A portion of the reference solution, which contained .06 mg. fluoride per 50 ml., was placed in the first cuvet; the three standard solutions were placed in the other three cuvets. The solutions in the cuvets were allowed to sit in the cell compartment of the Beckman DU spectrophotometer ten minutes before a reading was taken. The purpose of this waiting period was to permit temperature equilibration. After this first reading the solutions were still left in the instrument and a second reading was taken after an interval of about seven minutes. This served as a check on the first reading. If the readings agreed within an absorbance of .001 they were considered satisfactory. If not, a third reading was taken. This whole process was repeated with a second set of cuvets while the first set was being rinsed with fluoride-free water, soaked in dilute nitric acid, rinsed again with fluoride-free water, rinsed with the next set of solutions to be determined, and filled with these solutions. Thus while the solutions in one set of cuvets were being subjected to temperature equilibration

in the instrument the other set of cuvets was being cleaned and filled with the next group of samples to be determined.

The DU is provided with a special "check" setting which provides an electrical circuit equivalent to that formed when the absorbance potentiometer is set at zero. The convenience of this setting was discarded in the present experiment. Instead the potentiometer circuit was left in throughout. The absorbance was set at .000 with the reference solution in the light path. After the absorbances of the other three solutions were read, the absorbance of the reference solution was read just as if it were an unknown. This gave a check on any fluctuations that might have occurred in the instrument. If the reading came within $\pm$ .001 of .000 it was assumed that no significant instrumental fluctuations had taken place. Operational errors have also been discovered by the above procedure. In at least one case the fact that the phototube positioning knob had been accidentally bumped during a series of determinations was discovered. In another case it was found that the cuvets had not been properly positioned when the original zero setting with the reference solution had been made.

After all the unknown solutions had been run the standards were checked in each set of cuvets. The average of the absolute values (i.e., regardless of sign) of the changes in absorbances of each of the three standard solutions in this final reading from the absorbances found in the initial reading are given in table III in the appendix. Twenty-eight runs are averaged. The standard deviations and the ranges of changes involved are also given in the table. An error of .001 in absorbance represents a fluoride error of about .00012 mg. fluoride per 50 ml. unknown. Therefore an error of .004 in absorbance represents

a fluoride error of about .005 mg. fluoride per 50 ml. unknown. Since most of the unknown samples contain from 50 to 200 times this amount the error here is seen to be negligible. This is especially so when it is compared with the error resulting from incomplete recovery of fluoride in the distillation process.

In some cases the absorbance of the unknown was lower than that of the .050 mg. standard. This was due to the fact that the unknown contained more fluoride than the standard. Such samples were determined by diluting a known volume of developed unknown with a known volume of developed standard zero fluoride solution, or by diluting a known volume of undeveloped unknown with a known volume of undeveloped zero fluoride standard and then adding 5 ml. each of reagents A and B (each 0.04 M in hydroxylamine hydrochloric acid) to 50 ml. of the mixture. The agreement of the two methods in the values found for the total milligrams of fluoride in samples was not particularly good: egs., .265 vs. .248, .127 vs. .119, .512 vs. .502. Again, however, the error is probably less in most cases than the error due to incomplete recovery in the distillation process.

Attempts were made to arrive at cell (cuvet) corrections by placing the same solution in all four cuvets, setting the absorbance to zero with the first cuvet in the light path, and reading the absorbances of the other cuvets. Water and developed solutions containing .05, .02, and .00 mg. fluoride per 50 ml. were used. The cell correction obtained seemed to depend on the solution used, but not in any consistant manner. Since the correction was rarely over .005 absorbance units it was disregarded.

Since the wavelength calibration of a spectrophotometer may be

off it is not safe to use a wavelength of maximum absorption as reported in the literature without first checking to see that the given wavelength setting holds for the instrument being used. Thus early in the project a spectral curve was run using various fluoride standards versus the Megregian reference solution. When a new instrument arrived a new spectral curve had to be run in the vicinity of the wavelength of maximum absorbance. When the wavelengths were recalibrated on this instrument, a spectral curve again had to be run. A curve also had to be determined for the Beckman B spectrophotometer when experiments were done with this instrument. Finally, when the Megregian reference solution was discarded in favor of a standard fluoride reference solution, another standard curve had to be run. This was due to the fact that the wavelength of maximum difference of absorbance between the fluoride solutions and the Megregian reference solution does not correspond to the wavelength of maximum absorbance of the fluoride solutions.

An attempt was made to prepare a single reagent combining reagents A and B in the presence of hydroxylamine hydrochloride, but a considerable amount of the dye precipitated out, presumably due to the acidity of the solution and the concentration of the reagents. The structure of eriochrome cyanine R (See figure I in the appendix.) is such that a decreased solubility in acid solution would be expected.

RESULTS

Distillation Recovery.

In an attempt to determine whether or not all the fluoride had come over in the first 100 ml. of distillate, second and sometimes third and fourth 100 ml. portions of distillate were collected. Tests involving the distillation of sodium fluoride by itself showed very close to 100% recovery in the first 100 ml.--.0496 out of .0500 mg. of fluoride in each of two cases, and .098 out of .100 mg. of fluoride in each of two other cases--and very little if any fluoride in the second 100 ml. However, when second 100 ml. portions of distillate from urine and stool ash samples were collected they almost invariably contained significant amounts of fluoride. The average amount for 46 feces samples, expressed as percent of the amount found in the first 100 ml., was 8% with a standard deviation of 6%. The range was from "-3.7%" to 32%. For 22 urine samples the average amount was 10% with a standard deviation of 7%. The range was from "-0.76%" to 25%. Third and fourth 100 ml. portions of distillate collected also contained significant amounts of fluoride, sometimes even more than did the second 100 ml.

The presence of fluoride in distillate collected after the first 100 ml. may probably be attributed in large degree to entrapment of fluoride in the copious silver chloride precipitate formed on addition of silver perchlorate to the mixture of ashed sample and perchloric acid in the distillation vessel. As previously mentioned, it is necessary to precipitate the chloride in order to prevent it from distilling over as HCl and interferring with the determination. A fair amount of silver chloride in the form of small clumps is carried

by the bubbles formed above the boiling liquid to the upper walls of the distillation vessel and adheres there. Also, sometimes large hard-to-break-up lumps of silver chloride form at the bottom of the distillation vessel. It is suspected that the fluoride found in 100 ml. portions of the distillate subsequent to the first 100 ml. is due to slow leaching of fluoride from these clumps of silver chloride on the still wall and at the bottom of the still. A larger amount of fluoride would be expected to be leached and to distil over into a given 100 ml. of distillate if some of the silver chloride on the walls were washed by the boiling solution back down into the stills, and/or if a large lump of silver chloride in the bottom of the still partially broke up.

In the other direction, significant amounts of fluoride sometimes remain in the stills following a distillation even after attempts to clean the stills. This carries over into the next distillate, thus tending to raise the results. The amount of fluoride thus carried over has been as much as .01 mg. following the distillation of a high fluoride sample. This has been shown by subsequent "still blank" determinations.

A few attempts were also made to study distillation recovery by adding varying known amounts of fluoride to equal portions of a homogenized pool of samples. For comparison, some portions would have no fluoride added. All portions would then be taken through the drying, ashing, distillation, and determination processes. Then the fluoride found in given portions to which a known amount of fluoride had been added would be compared with the amount of fluoride present in portions to which no fluoride had been added. If recovery were 100% the difference between the two would be the amount of

fluoride added to the given portion. The results of these few studies were unduly inconsistent, presumably due to experimental mixups and to the necessity of redistillation in several instances. More work needs to be done along these lines before meaningful results can be obtained. The one thing that was ascertained was that not all the fluoride came over in the first 100 ml., since appreciable amounts were found in the second and subsequent 100 ml. portions.

It can readily be seen from the above paragraphs that additional experimentation needs to be done on the method of isolation of fluoride.

Spectrophotometric Determination.

The present study (See figure III in the appendix.) and that of Megregian (44) showed the fluoride standard curve to be nearly linear in the range of fluoride concentration from .000 to .050 mg. fluoride per 50 ml. of solution. Therefore only a three point check--.000, .025, and .050 mg. fluoride per 50 ml. of solution--was made for each day's group of determinations. All measurements were made against a solution made up in large quantity containing .060 mg. of fluoride per 50 ml. and the proper amounts of reagents A and B. Since the higher the fluoride content the lower the absorbance, this produced a solution that would likely have an absorbance smaller than that of the .050 mg. standard even when different batches of reagents A and B were used in making up the standard. This reference solution apparently varied from day to day, thus producing inconsistent absorbances of the standards relative to it. Consistent results for the standard solutions could be obtained, however, by

subtracting the absorbance of the .050 mg. standard from the absorbance of each of the standards. This value was also subtracted from the absorbance readings of the unknowns to put them on the same scale. For a given batch of reagents A and B all the absorbance values found for each given standard relative to the .050 mg. standard were averaged to determine a point on the standard curve for that batch of reagents. Four such series of average values, along with their standard deviations, are given in table IV in the appendix.

Fluoride Excretion Patterns in Infants with Various Forms of Dietary Fluoride Supplementation.

The results of the study are summarized in tables V, VI, and VII (See appendix.) and displayed in graphical form in figure IV (See appendix.). In each case the average amount of fluoride in the feces per six-hour urine collection period is calculated by dividing the total amount of fluoride in all the feces collected during the forty-eight hour test period by eight, the number of urine collection periods. This is added to the amounts of fluoride in the urine during the various collection periods to obtain, at least approximately, the total amount of fluoride that would be expected to have been excreted per six-hour urine collection period had fecal output been continuous rather than intermittant.

Due to incomplete recovery in the distillation process it is probable that on the average the fluoride values listed in tables V, VI, and VII (See appendix.) should be increased by at least 10%.

It can be seen from the graph (figure IV in the appendix) that the form in which the fluoride was administered had no essential

effect on the excretion pattern. It will be noticed that there does seem to be an lag in fluoride excretion by patients given medication A (fluoride water); but this is not unexpected since this medication was given over a period of eight hours or more whereas medications A and B were given in one "lump" at the beginning of the experiment.

It should be noted that the percentage values shown in tables V and VII and in figure IV are based on the amount of supplementary fluoride administered, and do not take into account any fluoride that may have been present in the normal diet. Unfortunately no base-line samples were collected. However, one of the reasons that the Plymouth group was selected for the study was that the fluoride content of the water supply there was negligible.

Duplicate portions of most of the dried fecal samples and a few of the dried urine samples were subjected to the ashing, distillation, and determination processes. A comparison of the results obtained with these duplicate samples is shown in tables VIII and IX in the appendix. Values for samples with which redistillations or other anomaly producing factors were known to be involved are not included in the tables. In order to put the data from the various pairs of determinations on a common basis, the percent deviation of the individual values from their average was calculated for each pair of duplicates. From the information given in table VIII it can be seen that the range of deviation from the average for the sixteen stool samples listed is from 0.0% to 6.8%. The mean of the deviations of these duplicate samples from their averages can be calculated to 2.1%. Table IX reveals that with four urine samples listed the range of deviations from the average is from 0.1% to 3.6%. The mean of these deviations is 1.8%.

SUMMARY AND CONCLUSIONS

The fluoride content of urine and feces samples which had been collected from infants during consecutive time intervals after the adminstration of fluoride in various forms was measured. Fluoride when ingested as a constituent of the Upjohn 47-M liquid vitamin preparation apparently shows a nearly identical excretion pattern--and, presumably, a nearly identical retention pattern--in infants as it does when administered as plain sodium fluoride, either in water solution or as a tablet.

Techniques in various stages of the analytical procedure were established which improved the reproducibility of the results. The most important of these were improvements made on the colorimetric determination of fluoride as outlined by Megregian--especially when the Beckman DU spectrophotometer is employed as the measuring instrument--by substituting a developed standard fluoride solution for the reference solution suggested by Megregian, and by allowing the solutions to undergo temperature equilibration in the cell compartment of the spectrophotometer before taking the measurements.

The total range of fluoride observed in the unknown samples studied was from 0.00 mg. to 0.37 mg. Measurements on portions of distillates collected after the fluoride had supposedly been isolated from the distilland for determination indicated that on the average at least 10% of the total fluoride content of the samples was not recovered during the utilization of the procedure herein described. Thus it can be seen that more work needs to be done on methods of quantitative separation of fluoride from other constituents of the ashed biological materials.

TABLE I.

Changes in absorbance of various fluoride solutions relative to Megregian (44) reference solution between successive readings of the same solutions. The samples are left in the cell compartment for the entire series of readings.

Conc. (mg. F per 50 ml.)	1st reading	2nd reading	3rd reading	4th reading	5th reading	6th reading
0.01	.477 ± .002	.472	.471	.470	.469	.468
0.02	.410 ± .003	---	.392	.392	.390	.388
0.03	.334	---	.328	.325	.324	.323

TABLE II.

Changes in absorbances of fluoride solutions calculated relative to the solution containing 0.03 mg. F/50 ml. by subtracting its values as listed in table I from the values listed for the other fluoride solutions.

Conc. (mg. F per 50 ml.)	1st reading	2nd reading	3rd reading	4th reading	5th reading	6th reading
0.01	.143	---	.143	.145	.145	.145
0.02	.067	---	.064	.067	.066	.065
0.03	.000	---	.000	.000	.000	.000

TABLE III.

Averages in twenty-eight runs of the changes in absorbances of each of the three standard solutions relative to the .060 mg. F reference solution before and after the various series of determinations.

Standard (mg. F/50 ml.)	Average Change	Range
.050	.003 ± .002	.000 to .007
.025	.004 ± .004	.000 to .014
.000	.004 ± .003	.000 to .009

TABLE IV.

Averages in several series of determinations of the absorbances of various fluoride standard solutions vs. the standard solution containing .050 mg. fluoride per 50 ml. The figures following the absorbance values are the standard deviations. The different series were necessary due to use of different batches of reagents A and B and/or to other changes, such as using a different pipet in place of a broken one.

Series:	A (9 det'ns.)	B (6 det'ns.)	C (18 det'ns.)	D (10 det'ns.)
.050 mg. F/50 ml.	.000	.000	.000	.000
.025 mg. F/50 ml.	.202 ± .003	.214 ± .004	.201 ± .004	.197 ± .002
.000 mg. F/50 ml.	.442 ± .003	.458 ± .005	.436 ± .006	.420 ± .003

TABLE V.

Results of the Plymouth fluoride study.

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
A-1-a:	6	.0571	.0919	.1490	.149	---	---
	12	.0646	"	.1565	.306	---	---
	18	.0839	"	.1758	.481	---	---
	24	.0761	"	.1680	.649	---	---
	30	.0664	"	.1583	.808	---	---
	36	.0484	"	.1403	.948	---	---
	42	.0280	"	.1199	1.068	---	---
	48	.0496	"	.1415	1.209	---	---
A-1-b:	(Rejected, since some Med. A was lost).						

[1] Code for patient and test period involved. A-1, A-2, and A-3 refer to three patients who received Med. A. A-1-a refers to the second test period for patient A-1. Similar coding is used for patients receiving Med. B and Med. C.

[2] Hours at the end of urine collection periods.

[3] Mg. F^- in urine excreted during the 6 hour collection periods.

[4] Average mg. F^- in feces per 6 hour urine collection period; calculated by dividing the total F^- in all the feces samples involved by 8, the number of urine collection periods.

[5] Total mg. F^- per 6 hour collection period.

[6] Cumulative total mg. F^-.

[7] Percentage of F^- per 6 hour urine collection period (mg. excreted (V) x 100/2 mg.) for A and C; (mg. excreted (V) x 100/1 mg.) for B.

[8] Cumulative % excretion. (VI x 100/2 mg.) for A and C; (VI x 100/1 mg.) for B.

TABLE V (cont'd.).

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
A-2:	6	.0696	.0684	.1379	.138	---	---
	12	.147	"	.2154	.353	---	---
	18	.0770	"	.1454	.499	---	---
	24	.253	"	.3214	.820	---	---
	30	.0803	"	.1487	.969	---	---
	36	.108	"	.1764	1.145	---	---
	42	.0332	"	.1016	1.247	---	---
	48	.0365	"	.1049	1.352	---	---
A-3:	6	.0804	.0436	.1240	.124	---	---
	12	.285	"	.3286	.453	---	---
	18	.309	"	.3526	.805	---	---
	24	.147	"	.1906	.996	---	---
	30	.0810	"	.1246	1.120	---	---
	36	.0748	"	.1184	1.239	---	---
	42	.0354	"	.0790	1.318	---	---
	48	.0394	"	.0830	1.401	---	---
Ave. of A-1-a, A-2, and A-3:	6	---	---	.137	.137	6.8	6.8
	12	---	---	.234	.371	11.7	18.6
	18	---	---	.225	.595	11.2	29.8
	24	---	---	.227	.822	11.3	41.1
	30	---	---	.144	.966	7.2	48.3
	36	---	---	.145	1.111	7.2	55.6
	42	---	---	.100	1.211	5.0	60.6
	48	---	---	.110	1.321	5.5	66.0

TABLE V (cont'd.).

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
B-1-a:	6	.0558	.0305	.0863	.086	---	---
	12	.0553	"	.0858	.172	---	---
	18	.0656	"	.0961	.268	---	---
	24	.0300	"	.0605	.329	---	---
	30	.0390	"	.0695	.398	---	---
	36	.0570	"	.0875	.486	---	---
	42	.0562	"	.0867	.572	---	---
	48	.0439	"	.0744	.647	---	---
B-1-b:	6	.0000	.0484	.0484	.048	---	---
	12	.1525	"	.2009	.249	---	---
	18	.0336	"	.0820	.331	---	---
	24	.0563	"	.1047	.436	---	---
	30	.0367	"	.0851	.521	---	---
	36	.0257	"	.0741	.595	---	---
	42	.0478	"	.0962	.691	---	---
	48	.0309	"	.0793	.771	---	---
B-2-a:	6	.0257	.0468	.0725	.072	---	---
	12	.0401	"	.0869	.159	---	---
	18	.0269	"	.0737	.233	---	---
	24	.0172	"	.0640	.297	---	---
	30	.0364	"	.0832	.380	---	---
	36	.0400	"	.0868	.467	---	---
	42	.0269	"	.0737	.541	---	---
	48	.0216	"	.0684	.609	---	---

TABLE V (cont'd.).

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
B-2-b:	6	.107	.0320	.139	.139	---	---
	12	.107	"	.139	.278	---	---
	18	.0100	"	.0420	.320	---	---
	24	.0537	"	.0857	.406	---	---
	30	.0460	"	.0780	.484	---	---
	36	.0513	"	.0833	.567	---	---
	42	.0292	"	.0612	.628	---	---
	48	.0309	"	.0629	.691	---	---
B-3:	6	.217	.0312	.2482	.248	---	---
	12	.133	"	.1642	.412	---	---
	18	.0344	"	.0656	.478	---	---
	24	.0743	"	.1055	.584	---	---
	30	.0629	"	.0941	.678	---	---
	36	.0686	"	.0998	.777	---	---
	42	.0125	"	.0437	.821	---	---
	48	.0621	"	.0933	.914	---	---
B-4:	6	.116	.0468	.1628	.163	---	---
	12	.0796	"	.1264	.289	---	---
	18	.0707	"	.1175	.407	---	---
	24	.0411	"	.0879	.495	---	---
	30	.0328	"	.0796	.574	---	---
	36	.0637	"	.1105	.685	---	---
	42	.0157	"	.0625	.747	---	---
	48	.0427	"	.0895	.837	---	---

TABLE V (cont'd.).

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
Ave. of B-1-a, B-1-b, B-2-a, B-2-b, B-3, and B-4:	6	---	---	.126	.126	12.6	12.6
	12	---	---	.134	.260	13.4	26.0
	18	---	---	.079	.339	7.9	33.9
	24	---	---	.085	.424	8.5	42.4
	30	---	---	.082	.506	8.2	50.6
	36	---	---	.090	.596	9.0	59.6
	42	---	---	.071	.667	7.1	66.7
	48	---	---	.078	.745	7.8	74.5
C-1-a:	6	.310	.0658	.3758	.376	---	---
	12	.367	"	.4328	.809	---	---
	18	.0807	"	.1465	.955	---	---
	24	.177	"	.2428	1.198	---	---
	30	.0550	"	.1208	1.319	---	---
	36	.0569	"	.1227	1.441	---	---
	42	.0286	"	.0944	1.536	---	---
	48	.102	"	.1678	1.704	---	---
C-1-b:	(Rejected, since collection was incomplete).						
C-2:	6	.208	.0529	.2609	.261	---	---
	12	.186	"	.2389	.500	---	---
	18	.069	"	.1219	.622	---	---
	24	.106	"	.1589	.781	---	---
	30	.0592	"	.1121	.893	---	---
	36	.0755	"	.1284	1.021	---	---
	42	.04545	"	.0984	1.120	---	---
	48	.0534	"	.1064	1.226	---	---

TABLE V (cont'd.).

I[1]	II[2]	III[3]	IV[4]	V[5]	VI[6]	VII[7]	VIII[8]
C-3:	6	.140	.0582	.1982	.198	---	---
	12	.219	"	.2772	.475	---	---
	18	.175	"	.2332	.708	---	---
	24	.0574	"	.1156	.824	---	---
	30	.0561	"	.1143	.938	---	---
	36	.0630	"	.212	1.060	---	---
	42	.0124	"	.0706	1.130	---	---
	48	.0428	"	.1010	1.231	---	---
Ave. of C-1-a, C-2, and C-3:	6	---	---	.278	.278	13.9	13.9
	12	---	---	.316	.595	15.8	29.8
	18	---	---	.167	.762	8.4	38.1
	24	---	---	.172	.934	8.6	46.7
	30	---	---	.116	1.050	5.8	52.5
	36	---	---	.124	1.174	6.2	58.7
	42	---	---	.088	1.262	4.4	63.1
	48	---	---	.125	1.387	6.2	69.4

TABLE VI.

SUMMARY[1]: Total mg. fluoride excreted in 48 hours.

	Urine	Stool	Urine & Stool
A-1-a	.474	.735	1.209
A-1-b	---	---	---
A-2	.805	.547	1.352
A-3	1.052	.349	1.401
A(Ave.)	.777	.544	1.321
B-1-a	.403	.244	.647
B-1-b	.383	.388	.771
B-2-a	.235	.374	.609
B-2-b	.435	.256	.691
B-3	.664	.250	.914
B-4	.463	.374	.837
B(Ave.)	.430	.314	.745
C-1-a	1.177	.527	1.704
C-1-b	---	---	---
C-2	.803	.423	1.226
C-3	.765	.466	1.231
C(Ave.)	.915	.472	1.387

[1] Tables VI and VII contain summaries of some of the pertinent data found in table V. The coding (A-1-a, etc.) is the same as in table V.

TABLE VII.

SUMMARY[1]: Percent fluoride excreted in 48 hours.

	Urine	Stool	Urine & Stool
A-1-a	23.7	36.7	60.4
A-1-b	---	---	---
A-2	40.2	27.4	67.6
A-3	52.6	17.4	70.0
A(Ave.)	38.8	27.2	66.0
B-1-a	40.3	24.4	64.7
B-1-b	38.3	38.8	77.1
B-2-a	23.5	37.4	60.9
B-2-b	43.5	25.6	69.1
B-3	66.4	25.0	91.4
B-4	46.3	37.4	83.7
B(Ave.)	43.0	31.4	74.5
C-1-a	58.8	26.4	85.2
C-1-b	---	---	---
C-2	40.2	21.2	61.3
C-3	38.2	23.3	61.6
C(Ave.)	45.8	23.6	69.4

[1] Tables VI and VII contain summaries of some of the pertinent data found in table V. The coding (A-1-a, etc.) is the same as in table V.

TABLE VIII.

Comparison of amount of fluoride found for total samples using two different sample portions: Stool samples[1].

I[2]	II[3]	III[4]	IV[5]	V[6]
.125	.127	.126	.001	0.8
.0632	.0662	.0647	.0015	2.3
.569	.565	.567	.002	0.4
.276	.273	.2745	.0015	0.5
.244	.241	.2425	.0015	0.6
.102	.094	.098	.004	4.1
.158	.158	.158	.000	0.0
.0709	.0711	.0710	.001	0.1
.162	.156	.159	.003	1.9
.276	.253	.2645	.0115	4.3
.206	.192	.199	.007	3.5
.107	.118	.1125	.0055	4.9
.166	.145	.1555	.0105	6.8
.150	.149	.1495	.0005	0.3
.0611	.0586	.05985	.00125	2.1
.0357	.0348	.03525	.00045	1.3

[1] Samples with which redistillations or other anomaly producing factors were known to be involved are not included.

[2] Total fluoride in the sample as found by analysing the first sample portion.

[3] Total fluoride in the sample as found by analysing the second sample portion.

[4] Average value for total fluoride found in the sample by analysis of the two sample portions.

[5] Deviation of the individual values from the average value.

[6] Percent deviation of the individual values from the average value.

TABLE IX.

Comparison of amount of fluoride found for total samples using two different sample portions: Urine samples[1].

I[2]	II	III	IV	V
.200	.215	.2075	.0075	3.6
.0703	.0676	.06895	.00235	3.4
.0593	.0590	.05915	.00015	0.3
.0455	.0454	.04545	.00005	0.1

[1] Samples with which redistillations or other anomaly producing factors were known to be involved are not included.

[2] The column headings are the same as in table VIII.

FIGURE I.

Eriochrome Cyanine R

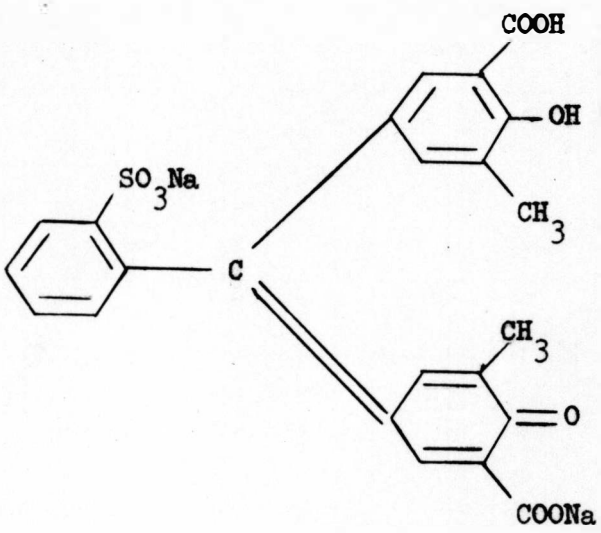


FIGURE II.

Heating of Distilled Water in the Cell Compartment of the Beckman DU Spectrophotometer

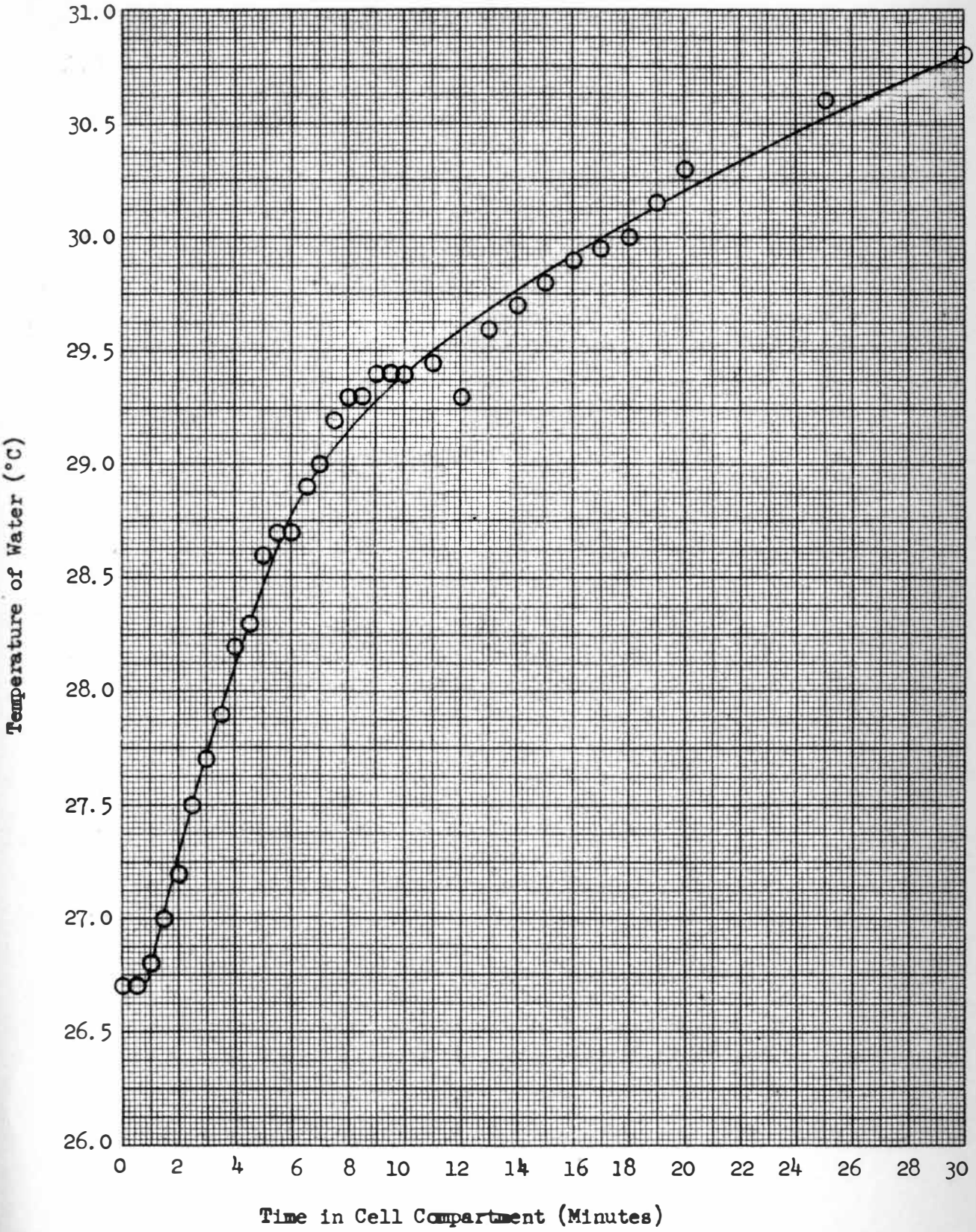


FIGURE III.

Fluoride Standard Curve

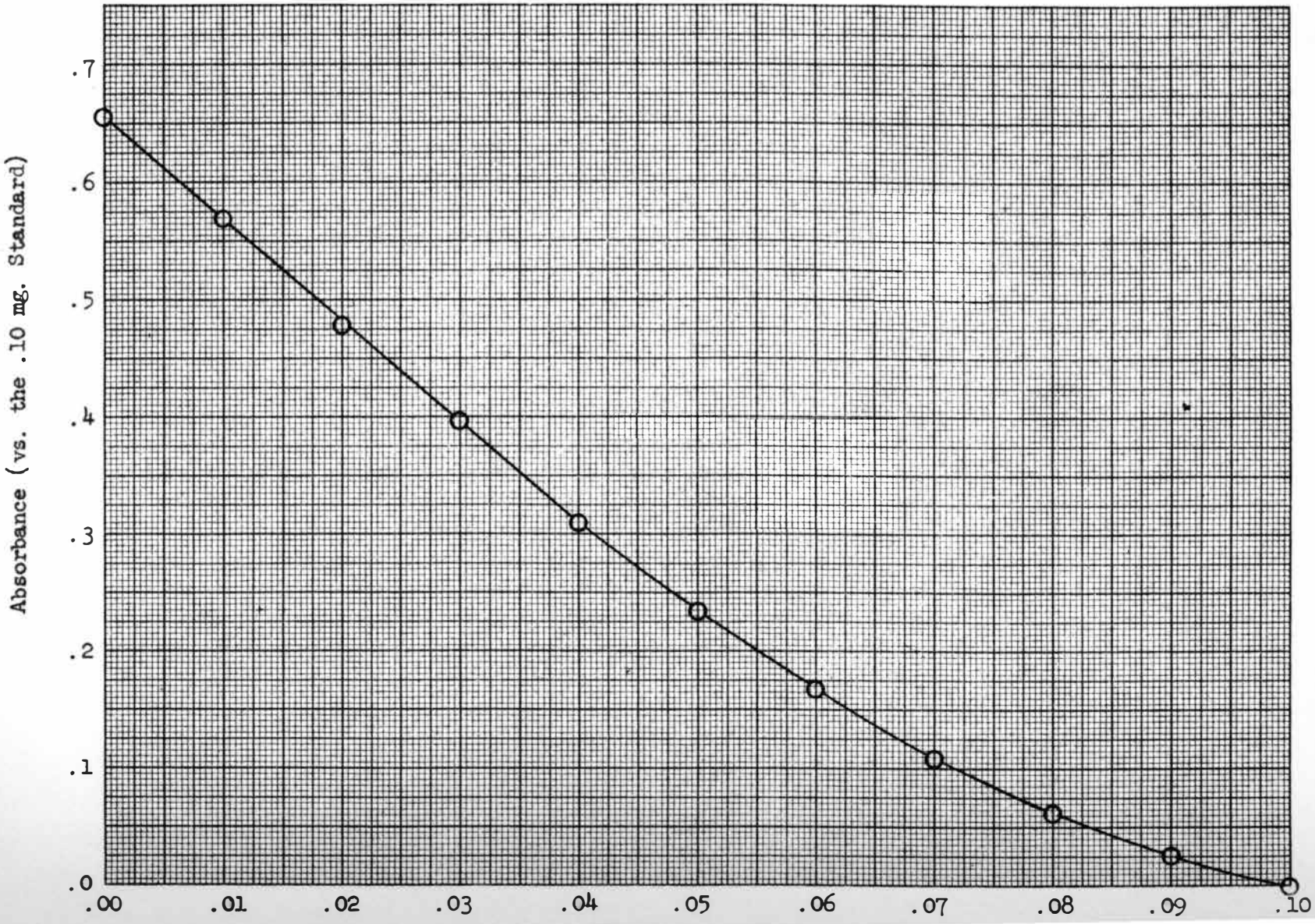
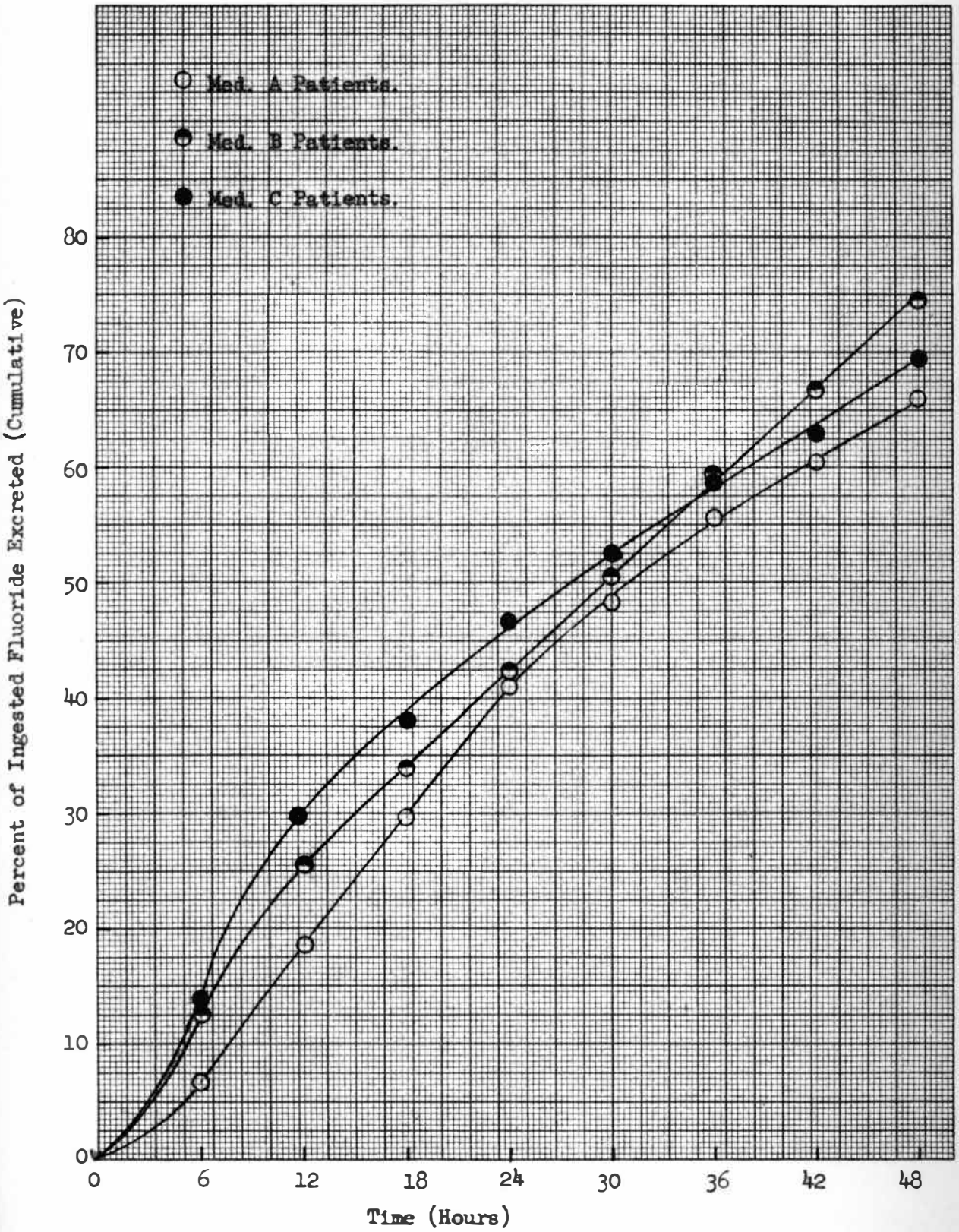


FIGURE IV.

Cumulative Fluoride Excretion in Urine and Feces
by Infants after Ingestion of Supplementary Fluoride



BIBLIOGRAPHY

1. Armstrong, W. D., Ind. Eng. Chem., Anal. Ed. 8, 384 (1936); C.A. 30, 7489[9].

2. Armstrong, W. D., J. Am. Chem. Soc. 55, 1741 (1933); C.A. 27, 2397.

3. Barr, G. and Thorogood, A. L., Analyst 59, 378 (1934); C.A. 28, 5515[6].

4. Baud, C. A., Acta Histochem. (in French) 4, 117 (1957); C.A. 51, 18064c.

5. Clifford, P. A., J. Assoc. Official Agr. Chem. 23, 303 (1940); C.A. 34, 5186[7].

6. Clifford, P. A., J. Assoc. Official Agr. Chem. 24, 350 (1941); C.A. 35, 5575[2].

7. Clifford, P. A., J. Assoc. Official Agr. Chem. 25, 394 (1942); C.A. 36, 4609[6].

8. Cremer, H. D., and Voelker, W., Biochem. Z. 324, 89 (1952); C.A. 47, 12487i.

9. Crutchfield, W. E., Ind. Eng. Chem., Anal. Ed. 14, 57 (1942); C.A. 36, 985[5].

10. Cummins, J. E., and Higginson, W. A., J. Council Sci. Ind. Research 10, 158 (1937); C.A. 31, 7619[8].

11. Dahle, D., J. Assoc. Official Agr. Chem. 18, 194 (1935); C.A. 29, 4839[6].

12. Dahle, D., J. Assoc. Official Agr. Chem. 19, 228 (1936); C.A. 30, 4934[9].

13. Dahle, D., J. Assoc. Official Agr. Chem. 20, 505 (1937); C.A. 31, 8431[3].

14. Dahle, D., J. Assoc. Official Agr. Chem. 21, 208 (1938); C.A. 32, 5934[3].

15. Dahle, D., J. Assoc. Official Agr. Chem. 21, 435 (1938); C.A. 32, 8604[7].

16. Dahle, D., Bonnar, R. V., and Wichmann, H. J., J. Assoc. Official Agr. Chem. 21, 459 (1938); C.A. 32, 3983[5].

17. Dahle, D., Wichmann, H. J., and Bonnar, R. V., J. Assoc. Official Agr. Chem. 21, 468 (1938); C.A. 32, 8983[9].

18. Danilova, V. V., Trav. lab. biogeochim. acad. sci. URSS 7, 76 (1944); C.A. 41, 1575f.

19. Elsworth, F. F., and Barritt, F. F., Analyst 68, 298 (1943); C.A. 38, 2609.

20. Epars, L., Bull. schweig. Acad. med. Wiss. (in French) 8, 360 (1952); C.A. 47, 3385b.

21. Fabre, R., Truhaut, R., and Rouquetti, A., Compt. rend. 240, 226 (1955); C.A. 49, 8039d.

22. Feigl, F., and Krumholz, P., Mikrochem. Pregl Festschr., 77 (1929); C.A. 24, 2400.

23. v. Fellenberg, T., Mitt. Lebensm. Hyg. 28, 150 (1937); C.A. 32, 2476.

24. Foster, M. D., J. Am. Chem. Soc. 54, 4464 (1932); C.A. 27, 357.

25. Fryd, C. F. M., Food Manuf. 31, 236 (1956); C.A. 50, 13317c.

26. Garratt, D. C., and Moir, D. D., Analyst 70, 442 (1945); C.A. 40, 16051.

27. Gautier, A., and Clausmann, P., Ann. fals. 5, 329 (1912); Compt. rend. 154, 1469 (1912); C.A. 6, 29004.

28. Geriche, S., and Kurmies, B., Landw. Forsch. 3, 46 (1951); C.A. 45, 7467i.

29. Greeff, A., Ber. 46, 2511 (1913); C.A. 7, 3939.

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

31. Hardin, L. J., MacIntire, W. H., and Tubb, M. E., J. Assoc. Official Agr. Chem. 37, 552 (1954); C.A. 49, 97c.

32. Horwitz, W., ed., Official methods of analysis, Assoc. Official Agr. Chem., Washington, D.C., 1955; p. 417.

33. Huckabay, W. B, Welch, E. T., and Metler, A. V., Ind. Eng. Chem., Anal. Ed. 19, 154 (1947).

34. Ikert, B., Chem. Ztg. 63, 324 (1939); C.A. 33, 56241.

35. Ikert, B., Chem. Ztg. 63, 754 (1939); C.A. 34, 21512.

36. Kolthoff, I. M., and Stansby, M. E., Ind. Eng. Chem., Anal. Ed. 6, 118 (1934); C.A. 28, 26435.

37. Kraft, K., and May, R., Z. physiol. Chem. 246, 233 (1937); C.A. 31, 46878.

38. LeRoy, A., Bull. soc. ind. Rouen 37, 568 (1909); C.A. 4, 18757.

39. Lockwood, H. C., Analyst 62, 775 (1937); C.A. 32, 9991.

40. Majumdar, B. N., and Ray, S. N., Indian J. Med. Research 35, 323 (1947); C.A. 42, 5075i.

41. Malm, M. Arkiv Kemi, Mineral. Geol. 25A, No. 1, 187 pp. (1947) (in German); C.A. 43, 268a.

42. Mayrhofer, V. A., Schneider, C., and Wasitzky, A., Biochem. Z. 251, 70 (1932); C.A. 26, 5513.

42a. Mayrhofer, V. A., and Wasitzky, A., Biochem. Z. 204, 62 (1929); C.A. 23, 1921.

43. McClendon, J. F., and Foster, W. C., Ind. Eng. Chem., Anal. Ed. 13, 280 (1941); C.A. 35, 3719[5].

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

45. Merwin, H. E., Am. J. Sci. 28, 119 (1935); C.A. 3, 2919.

46. Miller, R. F., and Phillips, P. H., Anal. Chem. 25, 172 (1953); C.A. 47, 3385a.

47. Moiseev, S. V., Stomatologiya 1951, No. 1, 32; C.A. 45, 5750h.

48. Nagler, R., Western Michigan University, Personal communication.

49. Nichol, A. A., Benedict, H. M., Byrne, J. L., and McCarty, C. P., Proc. Air Pollution Control Assoc. 49, 18 (1956); C.A. 51, 3891g.

50. Noetzel, O., Z. Nahr. Genussm. 49, 31 (1925); C.A. 19, 1917[6].

51. Nommik, H., Acta Polytech., Chem. Met. Ser. 3, 7 (1953); C.A. 48, 4364c.

52. Paterno, E., Gazz. chim. ital. 49, 371 (1919); C.A. 14, 3386[9].

53. Remmert, L. F., Parks, T. D., Lawrence, A. M., and McBurney, E. H., Anal. Chem. 25, 450 (1953); C.A. 47, 5305e.

54. Schulz, A., Arch. Tierernähr. 3, 65 (1952); C.A. 48, 1455a.

55. Sharpness, G. R., and McCollum, E. V., J. Nutrition 6, 163 (1933); C.A. 27, 4562.

56. Smith, O. D., and Parks, T. D., Anal. Chem. 27, 998 (1955); C.A. 49, 11494d.

57. Steiger, G., J. Am. Chem. Soc. 30, 219 (1908); C.A. 2, 1104.

58. Tedesco, P. H., Univ. nacl. LaPlata 24, 195 (1952); C.A. 47, 3176c.

59. Thompson, T. G., and Taylor, H. J., Ind. Eng. Chem., Anal. Ed. 5, 87 (1933); C.A. 27, 2072.

60. Venkateswarlu, P., and Rao, D. N., Indian J. Med. Research 42, 197 (1954); C.A. 48, 13779c.

61. Wauters, M., Bull. soc. med. not. Brussels, 1909, 193; through Biochem. Centr. 8, 551 (1909); C.A. 4 363[2].

62. Wichmann, H. J. and Dahle, D., J. Assoc. Official Agr. Chem. 16, 612 (1933); C.A. 28, 431[3].

63. Wichmann, H. J. and Dahle, D., J. Assoc. Official Agr. Chem. 16, 619 (1933); C.A. 28, 431[6].

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

65. Winter, O. B., J. Assoc. Official Agr. Chem. 19, 359 (1936); C.A. 30, 7068[2].

66. Winter, O. B., J. Assoc. Official Agr. Chem. 20, 333 (1937); C.A. 31, 8440[8].

VITA

The author was born in Plymouth, Indiana on September 6, 1931. He attended John Adams High School in the same state in the city of South Bend. He received a B.A. in Chemistry from Kalamazoo College in June of 1955. He then taught at the secondary level for three and one-half years followed by one-half a year of experience as a laboratory technician at Dr. Norman Beatty Memorial Hospital at Westville, Indiana. His fall term work on the M.A. degree in Chemistry began at Western Michigan University in the fall of 1959. He held a Graduate Teaching Assistantship during his first year there, and an Upjohn Research Grant and a National Institutes of Health Grant during his second year. He entered Purdue University under a Graduate Research Assistantship in the fall of 1961 to work toward a Doctorate in Biochemistry.